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STUDIES ON THE UTILIZATION OF AMINO ACIDS AS NITROGEN AND CARBON SOURCES FOR THE AEROBIC GROWTH OF YEAST

I. THE INFLUENCE OF GLUCOSE ON YEAST GROWTH WITH AMINO ACIDS AS NITROGEN DONORS

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ABSTRACT

The utilization of L-glutamic acid, L-aspartic acid, L-alanine, L-isoleucine, L-valine, L-phenylalanine and ammonia for the aerobic growth of the yeast strains *Torulopsis dattila* and *Saccharomyces cerevisiae* Hansen was investigated.

Torulopsis yeast utilizes all the amino acids for growth both in the absence and in the presence of glucose, but isoleucine, valine and phenylalanine were utilized very poorly without the addition of glucose.

The saccharomyces strain can use all of the amino acids for growth when glucose is present; in its absence only alanine, glutamic acid and aspartic acid can produce growth.

The relative degree of the utilization of the different amino acids is the same under optimal and suboptimal growth conditions.

Evidence is presented in favour of the hypothesis that the availability of the amino acid carbon is the growth-limiting factor when glucose is absent.

INTRODUCTION

Most yeast strains can use ammonium salts for aerobic growth in the presence of a suitable carbon source. Yeasts growing under conditions found in nature most probably derive at least part of their nitrogen requirements from amino acids occurring free or produced by the hydrolysis of the proteins found in the natural growth media. The ability of yeasts belonging to different strains to use amino acids for growth has therefore been made the subject of repeated investigations. Thorne[1] demonstrated that, in the presence of glucose, a number of amino acids can sustain growth and that their efficiency was, on the average, about 60% of that of ammonium salts, with the exception of glutamic and aspartic acids, which equalled or even exceeded ammonium salts with respect to the growth achieved under comparable conditions. Schultz and Pomper[2] reported similar experiments undertaken with a considerable number of torula and saccharomyces strains and found that some amino acids are used as both nitrogen and carbon donors, whereas others were not capable of utilization in the absence of an additional carbon source. The availability of a given amino acid for yeast growth was generally assumed to be determined by

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the presence of suitable mechanisms leading to its deamination, whereas the availability of its nitrogen-free carbon residue was less thoroughly investigated, owing to the fact that in most experiments glucose was supplied in adequate quantities. Since all the work mentioned was of a qualitative or at most semi-quantitative nature, the need was felt for a more thorough investigation of the utilization pattern of amino acids by yeasts. Experiments were therefore devised to determine quantitatively the relation of substrate uptake to growth under different conditions. For reasons of analytical accuracy the yeasts had frequently to be grown under suboptimal growth conditions, i.e. with small substrate concentrations. A preliminary investigation had therefore to be made on the influence of different glucose and amino acid concentrations on the availability of the latter for growth.

MATERIALS AND METHODS

All experiments reported in this and in the following paper were performed using two yeast strains commonly used in Israel for wine fermentation. One strain was isolated from locally grown grapes and was identified as a subspecies of *Torulopsis dattila* (Kluyver) Lodder. The other strain was isolated from dried grapes imported from Cyprus and identified as *Saccharomyces cerevisiae* Hansen.

Growth media

All growth media were prepared by standard bacteriological technique and were made to contain the following substances:

Salts (in mg/ml of growth medium, final concentration):

K_2HPO_4	0.5	NaCl	0.01
KH_2PO_4	0.5	$FeSO_4$	0.008
$MgSO_4 \cdot 7H_2O$	0.2	$MnSO_4 \cdot 4H_2O$	0.01

Growth factors (in μg/ml, final concentration):

Riboflavin	0.2	Calcium pantothenate	0.1
Thiamine HCl	0.1	Pyridoxin HCl	0.1
p-Aminobenzoic acid	0.1	Nicotinic acid	0.2
		Biotin	0.05

The media for the cultivation of the amounts of yeast cells needed for the growth experiments contained in addition 2% of peptone and glucose respectively. The same growth medium was used for the culturing of the yeast cells on slants. For this purpose the growth medium was made to contain 2% agar. The growth experiments were undertaken with the same medium, containing the ammonia, amino acid and/or glucose concentrations stated in the text.

Culturing methods

Both yeasts were kept as slant cultures on agar. The yeasts were allowed to grow for 24 hours at 30°C and the slant tubes were then kept at 2°. Subcultures were made bi-weekly. Larger quantities of yeast were grown by transferring the cells grown on one or two slants to 250 ml Erlenmeyer flasks containing 100 ml of the glucose-peptone growth medium, incubating with shaking for 24 hours at 30°. The cells were harvested by centrifuging, washed three times with distilled water and used for the preparation of the yeast suspensions for the growth experiments as described below. Larger quantities of yeast cells were produced by inoculating 3–4 litres of basic growth medium with 100 ml of growth medium, previously inoculated with two slants and incubated at 30° for 24 hours. Growth was then allowed to proceed for 24 hours at 30° with vigorous aeration. The yeast cells thus grown were then centrifuged off and washed as described.

Growth experiments

Cell growth was determined turbidimetrically in a Coleman spectrophotometer at 700 mμ, using Coleman standard test tubes (19 mm inner diameter) and 10 ml of yeast suspension.

Washed cells were suspended in water and the amount of suspension determined which, on dilution to 10 ml, gave an optical density reading of 0.170. This amount of yeast suspension was then pipetted into the number of test tubes necessary for the respective experiment, the necessary amounts of salts, growth factors, amino acids and glucose were added (as concentrated stock solutions) and the whole made up to 10 ml. All concentrations stated in this and the following paper refer to this final experimental mixture. The reliability of the turbidimetric measurements was tested by comparing them to cell counts and to the amount of dry matter present, the latter being determined by standard techniques. Linear dependence of turbidity on cell count and dry matter was observed for optical density values up to 0.70. Suspensions showing higher optical density values were suitably diluted before taking final measurements. A suspension of torulopsis yeast in 10 ml of growth medium having an optical density value of 0.170 corresponded to a cell count of 1.3×10^4 cells/mm^3 and the analogous suspension of saccharomyces cells contained 3×10^3 cells/mm^3. Suspensions of both strains with optical density of 0.170 corresponded to 60 μg of dry yeast cells/ml. The experimental mixtures prepared as described were incubated at 30° for 9 hours with vigorous shaking and removed every hour for the time necessary for the taking of the optical density measurements. All experiments were made in triplicate; the results reported are average values of at least three such experiments. The results were reproducible to within ± 5%.

RESULTS AND DISCUSSION

The nitrogen containing substrates tested were ammonia (as ammonium sulphate) L-glutamic acid, L-aspartic acid, L-alanine, L-valine, L-isoleucine and L-phenylalanine. The growth pattern, as determined by hourly measurements, was the same with all substrates at all concentrations and with both strains. The initial lag period of 3–3.5 hours was followed by logarithmic growth which lasted at least 12 hours. An experimental period of 9 hours' duration was therefore found to be sufficient to yield significant results and to obviate irregularities possibly arising from the depletion of substrates during an extended experimental period. The data presented in Figures 1a and 1b, which describe the influence of different nitrogen concentrations on growth therefore refer to the growth obtained at the end of the 9 hours' experimental period. The concentrations of the nitrogen-containing substrates varied between 10 and 250 μg of nitrogen per ml of growth medium.

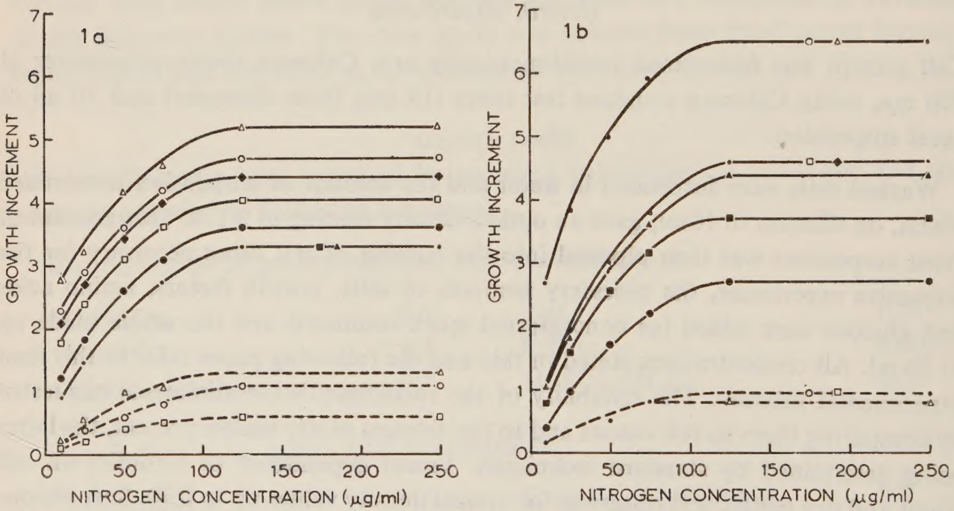

Figure 1

The dependence of the growth of the torulopsis strain (1a) and of the saccharomyces strain (1b) upon the ammonia and amino nitrogen concentration in the growth medium, in the presence of the constant glucose concentration of 20000 μg/ml (upper set of curves) and without glucose (lower set of curves)

Legend: The growth increment is defined as: $(B-A)/A$, where B is the amount of yeast cells at the end of the experiment and A the amount of yeast cells at its beginning

○ = glutamic acid, □ = alanine, ◆ = ammonium sulphate, ● = isoleucine, △ = aspartic acid, ▲ = valine, ■ = phenylalanine

In one series of experiments the growth medium contained, in addition to the N-containing substrate, 2% (= 20,000 μg) of glucose per ml of growth medium, in another series no glucose at all was added.

Under both these sets of conditions and with both strains, the minimal nitrogen concentration necessary to produce optimal growth was found to be 125 μg per ml of growth medium, independently of the amino acid given. The shape of the amino acid concentration vs growth plots is that of typical absorption curves, describing the gradual saturation of an absorbing surface with substrate. An active absorption mechanism acting specifically towards preferred substrates and thereby causing enhanced growth seems therefore improbable. On the other hand, the complete independence of the nitrogen concentration necessary to produce optimal growth of the growth actually achieved, i.e. of the quantity of nitrogen utilized by the cell within a specified period, suggests the assumption that the initial step of substrate assimilation may be its absorption on the cell surface, which is then followed by the penetration through an "inner membrane" into the cell interior. The rate of the latter process would be determined by the concentration gradient set up between the substrate concentration in the medium and that inside the cell, which in turn would be dependent on the rate of substrate utilization.

In contrast to the uniformity of amino acid concentration necessary to produce optimal growth, the actual growth achieved with the different nitrogen-containing substrates varies considerably. The amino acids investigated in this study fall into two groups according to their ability to sustain growth: Glutamic acid, aspartic acid and alanine (subsequently termed "group 1 acids") and on the other hand valine, isoleucine and phenylalanine (group 2 acids). The difference between the two groups is apparent both in the presence of a considerable surplus of glucose in the medium and, still more, in its complete absence, when the group 2 acids are very slightly utilized by the torulopsis strain but not at all by the saccharomyces strain. This result was not unexpected in view of the fact that all the group 1 acids yield as deamination products well-known and readily available metabolic intermediates. It should be pointed out that the availability of amino acids for growth in the total absence of any supporting carbon donor is a direct proof for their unchanged absorption by the cell, since growth media containing α-ketoglutaric acid, oxaloacetic acid or pyruvic acid together with ammonium sulphate did not produce growth, the cell wall apparently being impermeable to these acids. A deamination reaction proceeding prior to absorption seems therefore most improbable. Since the group 2 amino acids which, when given alone, produce only slight growth or are totally unavailable can be readily utilized in the presence of glucose, it can be concluded that the main factor determining their availability is not the difference in nitrogen utilization, but that in the availability of the carbon residue. This can also readily be seen from the results of experiments in which the amino nitrogen concentration is held constant (at the optimum level of 125 μg/ml previously determined) and the quantity of glucose added is progressively increased from 6 to 20,000 μg/ml (Figures 2a and 2b).

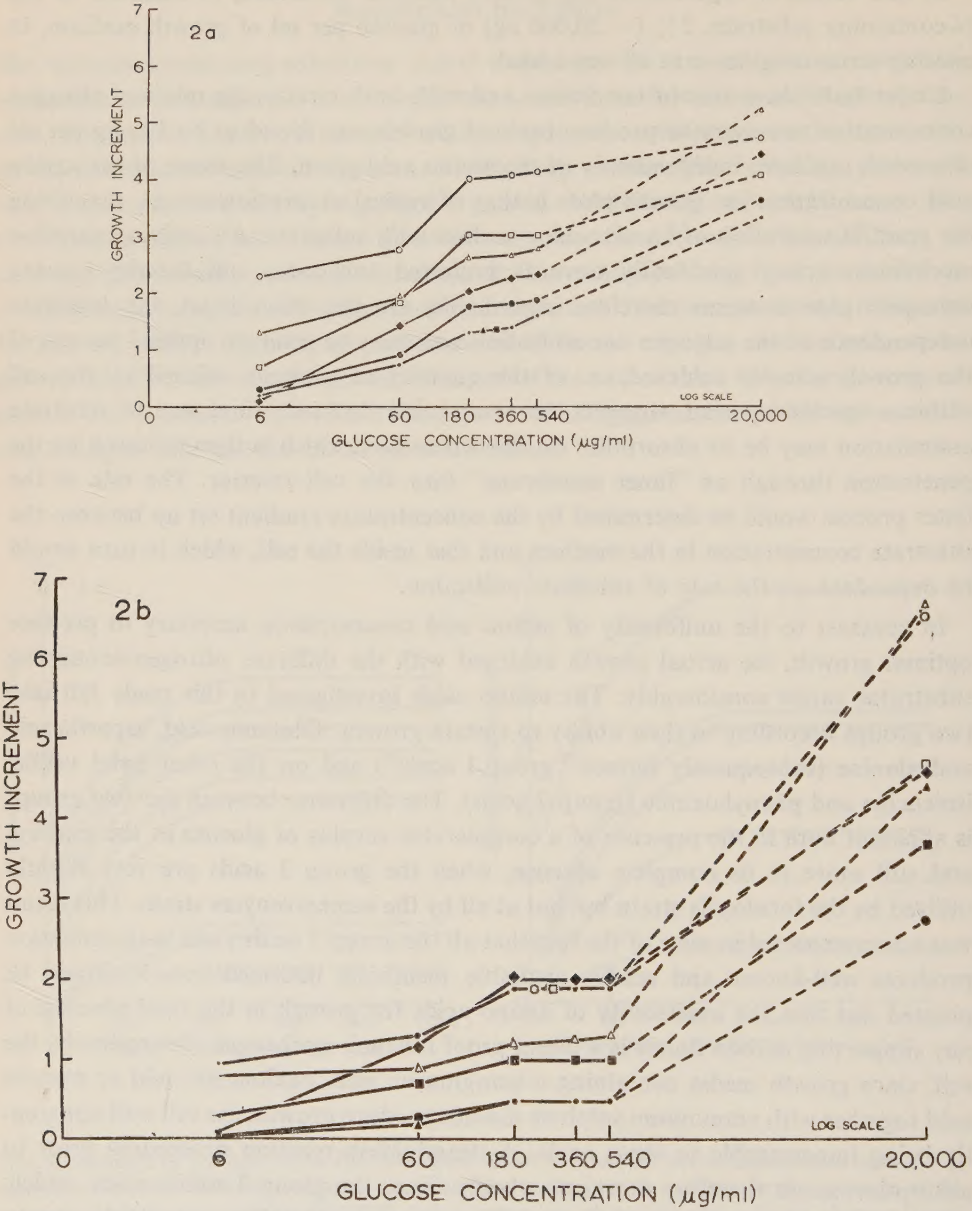

Figure 2

The dependence of the growth of the torulopsis strain (2a) and of the saccharomyces strain (2b) upon the concentration of glucose in the growth medium in the presence of the constant ammonia or amino acid nitrogen concentration of 125 μg/ml

Legend: See Figure 1

With the group 1 acids and glucose, growth is much higher at low glucose concentrations than that produced with the group 2 acids. This effect is due to the contribution made by the carbon residue of the amino acids to the carbon available for growth. It is therefore, of course, completely absent when ammonia is given. The plot of growth vs log glucose concentration are therefore not linear in the lower range of glucose concentrations. Linearity is observed only when ammonia is given instead of an amino acid, when no carbon other than that of glucose is introduced into the growth medium. Otherwise the influence of varying glucose concentrations on growth follows the same rule as that of the amino acids: The cell surface becomes saturated with glucose molecules at about 180 μg of glucose per ml of growth medium and this concentration is again the same with both strains. An increase of the glucose concentration up to 540 μg/ml cannot produce enhanced growth, but a further increase (to 20,000 μg/ml) brings about a very considerably enhanced growth. This unexpected observation shows that the absorption of glucose may be a composite process and determined by different mechanisms at lower and higher glucose levels. The absorption mechanism at lower glucose concentrations seems to be much the same as that found for the amino acids, but at higher glucose concentrations either the outer or the inner cell membrane seems to become more permeable to glucose, which is therefore being "forced through" and appears therefore in higher concentrations in the cell interior, thereby enhancing growth. This effect, which hitherto seems to have escaped the attention of most authors, possibly owing to the fact that in growth experiments devised to test the availability of N-containing substrates high glucose concentrations are employed routinely and exclusively, will be termed "forced growth". As mentioned before, the experiments reported so far had the aim to decide whether the availability of the different amino acids for growth depends on their concentration in the growth medium, i.e. whether the results obtained under suboptimal growth conditions can be regarded as significant and compared with those obtained under conditions of optimal growth. The relevant results are presented in Table I.

The concentrations of 180 μg of glucose/ml growth medium and of 26 μg N/ml were suboptimal, and the question has therefore to be discussed whether the results obtained under such conditions can be regarded as valid with respect to the amino acid utilization pattern observed.

It can readily be seen that the amino acid utilization pattern of both strains remains qualitatively the same regardless of whether the nitrogen concentration enables optimal growth or not, provided glucose is present. The difference in availability for growth between the group 1 acids and ammonia on the one hand and the group 2 acids on the other hand (roughly 1.5:1 in the torulopsis strain and 2:1 in the saccharomyces strain) remains also approximately the same when the substrate concentration of 25 μgN/ml and 180 μg glucose/ml is raised to 125 μg of nitrogen/ml and 20,000 μg of glucose/ml respectively. It can therefore be concluded that the results obtained with

TABLE I

Variation of growth increments with substrate concentration*

	Torulopsis dattila strain					*Saccharomyces cerevisiae* (Hansen) strain				
Glucose (in μg/ml)	20,000	180	180	0	0	20,000	180	180	0	0
N (in μg/ml)	125	125	25	125	25					
Nitrogen containing substrate										
Ammonium sulphate	4.42	2.29	1.53	—	—	4.62	2.00	0.95	—	—
Glutamic acid	4.72	4.10	1.53	1.25	0.44	6.50	1.90	1.06	0.95	0.53
Alanine	4.05	3.06	1.41	0.60	0.34	4.64	1.95	1.12	0.95	0.53
Aspartic acid	5.22	2.58	1.60	1.30	0.47	6.66	1.18	1.00	0.78	0.41
Isoleucine	3.60	1.80	1.18	0.25	0.09	2.72	0.57	0.53	0.05	—
Valine	3.32	1.29	0.94	0.18	0.18	4.42	0.67	0.65	0.05	—
Phenylalanine	3.32	1.42	1.30	0.32	0.24	3.71	1.06	1.06	0.05	—

* The growth increment is the increase of cells at the end of the experiment over the initial quantity and is calculated as: $(a-b)/b$, where a = the amount of yeast cells at the end of the experiment and b = the amount of yeast cells at the beginning of the experiment.

yeasts growing in a medium containing the suboptimal nitrogen concentration of 25 μg/ml together with 180 μg of glucose/ml can be regarded as valid for the discussion of the uptake of the different substrates by normally growing yeast. In the absence of glucose other results were to be expected, in view of the different availability of the carbon skeleton of the amino acids. It can be seen that the group 2 acids are not utilized at all by the saccharomyces strain in the absenec of glucose at both the lower and the higher nitrogen concentrations. Growth only sets in when glucose is added, since the carbon skeleton of all three acids of group 2 is apparently totally unavailable. That the presence of available carbon and not that of available nitrogen is the limiting factor in this case appears from the fact that no enhanced growth results from the increase of the amino acid nitrogen from 25 to 125 μg/ml when the glucose concentration remains constant. Full utilization of the group 2 acids by the saccharomyces strain is only achieved when "forced growth" conditions prevail. A similar effect can be observed with the group 2 acids which are utilized by the torulopsis strain in the total absence of glucose. Here again it can be seen that the small growth increment obtained with a nitrogen concentration of 25 μg/ml cannot be significantly increased when the nitrogen concentration is increased to 125 μg showing that the additional carbon introduced into the growth medium is not readily available. When glucose is added, however, an immediate growth response is obtained.

That the efficiency of the glucose carbon atoms with respect to the growth exceeds by far that of even the most efficiently utilized amino acid carbon residue can also be very strikingly demonstrated by comparing the growth increments obtained (in both strains) with 125 μg of amino acid nitrogen/ml in the absence of glucose and with 25 μg of amino acid nitrogen/ml in the presence of 180 μg of glucose/ml. The total carbon content at the higher nitrogen level is, for alanine, glutamic acid and aspartic acid, 320, 542 and 430 μg carbon/ml of growth medium respectively, whereas at the lower nitrogen level in the presence of 180 μg glucose/ml, the corresponding carbon concentrations are 136, 171 and 158 μg C/ml. One would therefore expect that growth at the higher amino acid concentration in the absence of glucose would considerably exceed that at the lower amino acid level with the addition of glucose, since in the former case the amounts of both nitrogen and carbon in the growth medium are conspicuously higher. Actually such an effect could never be observed. The addition of glucose to low amino acid concentrations was always more effective with respect to growth than the employment of large concentrations of even the group 1 amino acids alone, showing that none of the deamination products could be used by the yeast cell with the same efficiency as glucose.

The last factor to be taken into account when dealing with the utilization of amino acids for the growth of yeast cells is the possibility that different amino acids may produce yeast cells of different composition. Two different cases can be envisaged: (a) Apparent growth, as can be measured by turbidity measurements and dry matter determinations, but a lower nitrogen content the yeast cells produced in, and (b) the opposite effect, namely comparatively small growth but a higher nitrogen content in the cells produced. The first case would indicate that the amino acid carbon can be utilized, but not its nitrogen, whereas in the second case the cell body would be enriched with newly formed nitrogen compounds, but cannot somehow form other cell components necessary to produce growth. The production of new cells having a lower nitrogen content than the starting material could not be observed with amino acids or ammonia as substrates. It appeared that whenever growth occurred, the total nitrogen content of the cells remained almost unchanged. That, however, such an effect is possible is shown by the nitrogen content found in yeast cells grown in the total absence of a nitrogen-containing substrate. In this case the "growth" observed is accompanied by a corresponding decrease in total nitrogen, indicating that the increase in cells is achieved at the expense of the nitrogenous cell components which are diluted. Growth ceases when the original nitrogen content of the yeast cells has fallen to about 50% of its initial value.

The opposite effect, the enrichment of the cell interior with newly formed nitrogen-containing substances, was observed in one case, that of alanine, when given as sole substrate. In this instance the total nitrogen content of the yeasts belonging to both strains rose about 25% over its initial value.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Miss Sloof of the Yeast Division of the Centralbureau voor Schimmelcultures, Delft, Holland, for the isolation and identification of the yeast strains used in this work.

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STUDIES ON THE UTILIZATION OF AMINO ACIDS AS NITROGEN AND CARBON SOURCES FOR THE AEROBIC GROWTH OF YEAST

II. THE UPTAKE OF AMINO ACIDS, GLUCOSE AND OXYGEN DURING GROWTH

H. Meyer and M. Benziman

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ABSTRACT

The two yeast strains *Torulopsis dattila* and *Saccharomyces cerevisiae* Hansen were aerobically grown on media containing L-glutamic acid, L-aspartic acid, L-alanine, L-valine, L-isoleucine and L-phenylalanine respectively, with and without glucose.

All the amino acids were utilized by both strains in the presence of glucose. In its absence, valine, isoleucine and phenylalanine were used only poorly by the torulopsis strain and could not produce any growth with the saccharomyces yeast. In short term experiments, the uptake of amino acids and of glucose from the growth medium was determined and the uptake of oxygen and the liberation of CO_2 were measured and used for the calculation of respiratory quotients.

Uptake of amino acids in the presence of glucose was proportional to growth in the torulopsis strain, but exceeded growth in the saccharomyces strain. Both strains took up amino acids in excess of growth when glucose was absent. Amino acids which did not enable growth were also taken up from the medium.

Glucose was taken up by the torulopsis yeast in proportion to growth, with the exception of glutamic acid, which depressed glucose uptake considerably. The saccharomyces strain took up glucose in excess of growth in all cases. Oxygen was always taken up in proportion to growth.

Nitrogen uptake for unit growth was the same in both strains and with all amino acids in the presence of glucose. In its absence the amount of nitrogen absorbed varied and was found to be dependent upon the availability of the carbon residue of the amino acids for growth. Unwanted nitrogen was excreted into the medium; the nature of the nitrogen compounds excreted was not determined.Glucose uptake for unit growth was much larger in the saccharomyces strain and it varied in both strains with the availability of the amino acid carbon. Oxygen demand of the saccharomyces strain was about 30% larger than that of the torulopsis strain. In both strains the oxygen demand was fairly constant in the presence of glucose but much enhanced and varying when amino acids alone were given. It was roughly inversely proportional to the growth rate. The respiratory quotient approached unity only when ammonium sulphate with glucose was given. It was lower than unity when amino acids were present. Glucose and amino acids were oxidized by the torulopsis strain in the ratio of approximately 1:1 when given together, and in the ratio 1:4 by the saccharomyces strain. In the absence of glucose very low respiratory quotients were observed, and it was concluded that the utilization of the amino acid carbon residue may be accompanied either by the synthesis of carbohydrates or by the reincorporation of initially produced carbon dioxide.

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INTRODUCTION

It has been shown in the preceding paper that the ability of amino acids to sustain aerobic growth varies within wide limits, depending on the amino acid given. Some amino acids, such as glutamic and aspartic acids, equalled or even exceeded ammonium salts in their efficiency for the promotion of growth, whereas others, such as isoleucine, were utilized only poorly, in particular when given as the sole substrate.

The availability of the amino acids for growth may be dependent either on the presence of efficient deamination mechanisms, such as the Stickland[1] or Ehrlich[2] reaction systems, or it may be predominantly due to the addition of a suitable carbon source, like glucose. The growth-stimulating effect of glucose may be a catalytic one, as suggested by Schultz and his co-workers[3] or it may simply be due to the fact that glucose is a much more efficient carbon source than the amino acid carbon skeleton remaining after deamination. The latter hypothesis was in general confirmed by the growth experiments reported in the previous paper. The experiments on the relation of substrate uptake to growth presented in the following were devised to supplement the information gathered by growth experiments alone with data of a more detailed and quantitative nature.

EXPERIMENTAL

The yeast strains *Torulopsis dattila* and *Saccharomyces cerevisiae* and the methods for their cultivation have been described in the preceding paper.

All growth experiments lasted 9 hours. They were carried out in growth media containing 25 μg of amino or ammonia nitrogen and, when given, 180 μg of glucose in one ml of growth medium, which was made to contain 60 μg of yeast cells (calculated as dry matter) in one ml. In each experiment, 30 test tubes, each containing 10 ml of growth mixture, were incubated. Three tubes were removed at hourly intervals for the determination of growth by turbidity. The cells were then centrifuged off and the amounts of substrate(s) remaining in the supernatant were determined by the following methods:

Glucose was determined by the anthrone method described by Dimler et al[4], ammonia by the method of Johnson[5], a modified Nessler procedure, and amino nitrogen by the quantitative ninhydrin (colorimetric) method described by Meyer[6].

Total nitrogen determinations were carried out by standard Kjeldahl procedure.

Dry matter was determined by drying the yeast cells at 105° to constant weight. Oxygen consumption and carbon dioxide liberation were measured by Warburg's "direct method" (Umbreit)[7] using standard equipment. The volume of the reaction mixture having the same composition as the growth mixtures described above, was 4 ml. Measurements were taken hourly during the total experimental period of nine hours.

The extraction of the free amino acids from yeast cells was carried out, as recommended by Spiegelman et al[8], by extraction with hot water. The amino acids were separated by paper chromatography, using standard phenol and butanol-acetic acid systems. Duplicate sheets were run and one of them was developed with ninhydrin in the usual manner. Using this as reference, the spots of the other (undeveloped) sheet were cut out and used for the quantitative amino acid determination, which was carried out according to Meyer[6]. Turbidity measurements were carried out using a Coleman spectrophotometer and 19 mm i.d. tubes at 700 mμ. The same instrument was used for the colorimetric amino nitrogen determination by the ninhydrin method at 576 mμ.

RESULTS

Uptake of substrates

Uptake of nitrogen and carbon-containing substrates from the growth medium and of oxygen from the atmosphere was measured at hourly intervals during the growth experiments.

Two characteristic stages in the growth process of the two yeast strains investigated were chosen for graphical representation (Figures 1a and 1b for the torulopsis strain and Figures 2a and 2b for the saccharomyces strain): The end of the lag period, when growth amounts to 10% of the total, and the point of 50% of total growth, well within the exponential growth phase.

Oxygen is the only substrate which, in the presence of glucose and independently of the nature of the N-containing substrate, is taken up almost in exact proportion to growth by both strains and in both growth phases. Considerable difference between the two strains exists with regard to glucose uptake, which is proportional to growth only in the torulopsis strain. This strain, however, shows two notable exceptions: at 10% growth and with glutamic acid as the N-containing substrate, no glucose was taken up, and at 50% growth only 35% of the total amount of glucose taken up had disappeared from the medium. Alanine, on the other hand, causes a considerable excess uptake of glucose at both growth stages. Glucose uptake exceeds growth in the saccharomyces strain at both growth stages; at 10% growth, the effect is more pronounced with the group 2 amino acids* as substrates, but glucose uptake at 50% growth exceeds growth to approximately the same degree with all amino acids.

Amino acids in the presence of glucose are taken up by the torulopsis yeast either in proportion to or in very slight excess to growth at both growth stages. The uptake of amino acids by the saccharomyces strain under the same conditions considerably exceeds growth.

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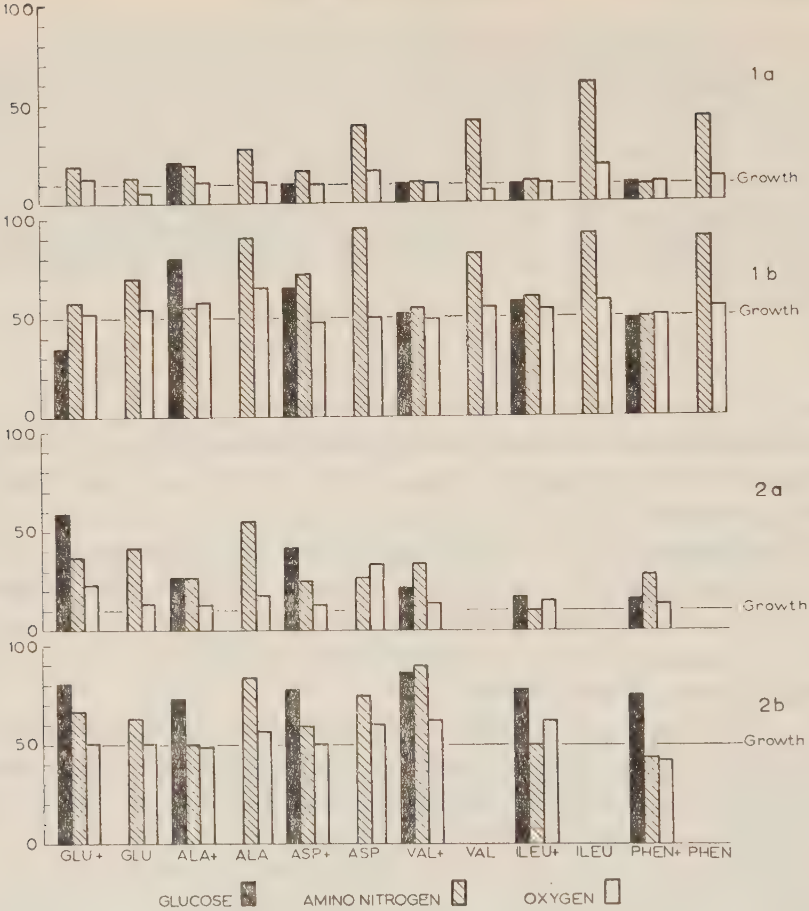
* Glutamic acid, alanine and aspartic acid, which can be efficiently utilized by the yeasts both in the presence and absence of glucose, are termed group 1 acids, whereas isoleucine, valine and phenyl-alanine, which are utilized in the presence of glucose, but are poorly or not at all available when given in its absence, are termed group 2 acids. For quantitative data the preceding paper should be consulted.



Figures 1 and 2

Figures 1a and 1b: Substrate uptake of the torulopsis strain (in % of the total) at 10 and 50% of total growth

Figures 2a and 2b: Substrate uptake (of the saccaromyces strain (in % of the total) at 10 and 50% growth.

(A + sign following the names of the amino acids denotes that they were given together with glucose; for substrate concentrations see text)

In the absence of glucose, amino acids are consumed by both strains in large excess over growth, with the sole exception of glutamic acid in the torulopsis strain.

Oxygen consumption remains proportional to growth in all cases and is not influenced by the absence of glucose.

Utilization of substrates for unit growth

Results comparing the efficiency of the different substrates in promoting growth are presented in Table I. The quantities of substrates necessary to bring about the production of one mg of yeast cells (calculated as dry matter), termed unit growth, are given in μg for solid substrates and μl for oxygen.

TABLE I

SUBSTRATE UPTAKE AND GROWTH

(Substrate uptake in μg substrate taken up for the growth of 1 mg dry yeast cells)

Substrate	*Torulopsis datilla*					*Saccharomyces cerevisiae*					
	Growth increment*	Nitrogen	Glucose	O_2 (μl)	RQ**	Growth increment*	Nitrogen	Glucose	O_2 (μl)	RQ*	RQ** theor.
Ammonium sulphate+ glucose	1.53	128	1960	720	1.03	0.95	124	3030	1140	0.97	
Glutamic acid+ glucose	1.53	115	1120	710	0.81	1.06	114	2800	1060	0.87	
Glutamic acid	0.44	306	—	1190	0.58	0.53	161		2190	0.38	1.00
Alanine+ glucose	1.41	118	1210	700	0.67	1.12	142	2640	1350	0.71	
Alanine	0.34	310		2000	0.37	0.47	202		29308	0.22	0.86
Aspartic acid+ glucose	1.60	132	1930	700	0.90	1.00	160	3040	1430	0.84	
Aspartic acid	0.47	338		1430	0.58	0.41	300		3430	0.44	1.14
Valine+ glucose	0.94	175	2480	700	0.80	0.65	118	4290	1350	0.83	
Valine	0.18	538		2700	0.44	0.05	1500		7000	0.13	0.75
Isoleucine+ glucose	1.18	157	1600	700	0.78	0.53	156	3980	1320	0.80	
Isoleucine	0.09	960		5190	0.33	0.05	2000		8000	0.12	0.75
Phenylalanine+ glucose	1.30	177	1720	750	0.76	1.06	202	2920	1220	0.76	
Phenylalanine	0.24	700		2700	0.41	0.05	1000		6000	0.19	0.86

* The growth increments are calculated as [(*Total amount of yeast at end of experiment* — (*Initial amount of yeast*)] / (*Initial amount of yeast*)

** Average values over the experimental period of 9 hours (see text)

*** The respiratory quotient theoretically required for the total oxidation of the respective amino acid

Oxygen demand

Oxygen uptake with all the nitrogen-containing substrates in the presence of glucose amount to about 700 μl for the production of unit growth in the torulopsis strain and is remarkably constant for all substrates, while the corresponding value in the saccharomyces strain is much higher (about 1350 μl) and deviates from the average in two cases: Ammonium sulphate and glutamic acid show the lower O_2 uptake of about 1100 μl. Oxygen uptake is vigorously stimulated when amino acids are given as sole substrates. Uptake by the saccharomyces strain is again much higher than that shown by the torulopsis strain. In the absence of glucose, which as the preferred substrate for respiration acts as an equalizing factor, the influence of the structure of the different amino acids on oxygen consumption is much more pronounced and causes wide divergences between the uptake values obtained, though no quantitative correlation between structure and oxygen uptake is apparent. Oxygen uptake, however, is in both strains significantly higher for the poorly utilized group 2 acids.

The determination of oxygen uptake and that of carbon dioxide production allowed the calculation of respiratory quotients. These were calculated using measurements taken every hour. The RQ values so obtained in the course of a nine-hour experiment differed but little (less than 5%). It was therefore possible to present average values for the whole experimental period (Table I). The most remarkable fact appearing from the results is that the respiratory quotient reaches its theoretical value for the oxidation of glucose (1.0) in only one case, that of ammonium sulphate with glucose in both strains.

The respiratory quotients obtained with all other substrates in both strains in the presence of glucose range from 0.67 (alanine + glucose, torulopsis strain) to 0.90 (aspartic acid + glucose, torulopsis strain)and are therefore of the order of magnitude or even lower than the theoretical RQ values of the corresponding amino acids when oxidized alone. (These are given for comparison in Table I, column 11). That the RQ values observed with the amino acid–glucose mixtures are composite values, resulting from the simultaneous oxidation of glucose and the amino acid, is clearly apparent from the fact that RQ values for growth mixtures containing amino acids as sole substrates are still very much lower. The lowest values, down to 0.12 (isoleucine in the saccharomyces strain) are generally obtained with the group 2 acids, though the distinction between the two groups is by no means clear, since, for example, the RQ values measured for alanine in both strains are exceptionally low. The percentage contribution of each of the substrates, glucose and amino acid, to the sum total of oxidized material was calculated from the RQ values obtained with the amino acids with and without glucose by the expression:

$$x = 100\,\frac{1 - A}{1 - B}$$

where x is the percentage contribution made by the amino acid to the total substrate oxidized, A is the respiratory coefficient measured in the presence of glucose and B that obtained in its absence. The results are given in Table II. The two strains are seen to differ in that the torulopsis strain oxidized about equal amounts of amino acids and glucose, while in the saccharomyces strain the contribution of glucose to the total amount of substrate oxidized is much larger and ranges from 60 to 80% of the total. This finding is in accord with the observation that the saccharomyces strain has a higher glucose demand and permits the conclusion that the lower contribution of the amino acids to the total oxidized material is rather due to the higher amounts of glucose consumed than to the lower amounts of amino acids taken up. It should be noted that the contribution made by aspartic acid to the total material oxidized in the torulopsis strain is exceptionally low, 23% compared with about 50% for all the other amino acids.

TABLE II

The substrates oxidized by the yeasts: the contribution made by the amino acids and by glucose, when simultaneously oxidized by the growing yeasts, calculated as per cent of total material oxidized

	Torulopsis dattila	*Saccharomyces cerevisiae*
Glutamic acid	45	21
Glucose	55	79
Alanine	47.5	37
Glucose	52.5	63
Aspartic acid	23	39.5
Glucose	76	60.5
Valine	43.5	19.5
Glucose	56.5	80.5
Isoleucine	32.5	23
Glucose	67.5	77
Phenylalanine	40.5	30
Glucose	59.5	70

Uptake of glucose

With regard to the quantities of glucose necessary to produce unit growth, the two yeast strains differ, the glucose demand of the saccharomyces strain being about one-third higher than that of the torulopsis strain. This observation closely parallels that on oxygen demand in which the two strains also differ by about the same percentage.

The availability of the amino acid carbon would be expected to manifest itself as a "glucose sparing action", diminishing the total glucose demand in proportion to the contribution made by the amino acid carbon to the carbon supply required by the growing cell. Such an effect can clearly be seen in the torulopsis strain, where the glucose required for unit growth is lowered about 40 to 60% when glutamic acid or alanine are given, when compared to that in the presence of ammonium sulphate. Isoleucine and phenylalanine slightly lower the glucose demand; aspartic acid has no glucose-sparing action and glucose uptake is considerably stimulated by valine. In the saccharomyces strain glutamic acid and alanine lower the glucose demand to only about 10% below that observed with ammonium sulphate, aspartic acid and phenylalanine are inactive and valine and isoleucine clearly stimulate glucose uptake.

Uptake of amino acids

The amount of nitrogen required for unit growth varies but little with the different N-containing substrates when glucose is present and is of the same order of magnitude in both strains. Nitrogen uptake for unit growth is, in the torulopsis strain, the same for ammonium sulphate and all the group 1 acids, with the group 2 acids as N-containing substrates, the N-demand is about 20% higher. In the saccharomyces strain only glutamic acid causes the same nitrogen demand as ammonium sulphate, all other amino acids cause the disappearance of larger amounts of nitrogen from the medium.

When amino acids are given as sole substrates, nitrogen demand is considerably higher and its dependence on the structure of the amino acid is much more apparent. All group 1 acids when given to the torulopsis strain approximately double the amount of nitrogen absorbed for unit growth; with the group 2 acids three (valine) to five (isoleucine) times the nitrogen disappear from the medium as in the presence of glucose. In the saccharomyces strain, glutamic acid, alanine and aspartic acid behave in a manner similar to that with the torulopsis strain, but all the group 2 acids are absorbed to a considerably higher degree despite the almost complete absence of growth.

The larger amounts of nitrogen absorbed in the absence of glucose do not, however, remain in the cell, but are for the greater part re-excreted into the medium. This appears from the results presented in Table III, which shows the amounts of nitrogen actually incorporated in the cell during growth, the amounts of *amino* nitrogen which disappear from the medium and the *total* nitrogen remaining in the medium at the end of the experiment. It can clearly be seen that a much larger quantity of amino nitrogen disappears from the medium than is incorporated in the cells. The amounts of *total* nitrogen incorporated are, however, the same as the total nitrogen taken up from the medium. The amino nitrogen initially absorbed has therefore been partly re-excreted during growth in the form of a nitrogen compound which does not react with ninhydrin.

It should be noted that in the saccharomyces yeast with the group 2 acids as substrates where practically no growth takes place, certain amounts of amino acids are absorbed and almost exactly the same quantity of non-amino nitrogen has been excreted, thus indicating a turnover reaction. Otherwise the amounts of nitrogen excreted are larger when amino acids alone are given (this fact can best be seen by comparing the values appearing in columns 4 and 8 in table III, where the amount of nitrogen excreted per microgram of nitrogen absorbed has been calculated).

TABLE III

Nitrogen balance experiments (amounts of nitrogen in μg)

	Torulopsis dattila					*Saccharomyces cerevisiae*				
Substrate	Total nitrogen incorporated	Amino nitrogen absorbed	Total nitrogen disappeared from medium	Total nitrogen excreted	G nitrogen excreted/g nitrogen consumed	Total nitrogen incorporated	Amino nitrogen absorbed	Total nitrogen disappeared from medium	Total nitrogen excreted	G nitrogen excreted/g nitrogen consumed
Glutamic acid 1*	75	106	76	30	0.29	51	72	52	20	0.28
2	31.5	81	30.9	50.1	0.62	26	39	26.5	13.5	0.25
Alanine 1	68	100	66.2	37.8	0.38	55	95	55	40	0.42
2	21	57	20.8	36.2	0.64	26.5	57	26	29	0.51
Aspartic 1	13.4	191	131	60	0.31	48	96	46.9	49.1	0.51
2	90.8	138.3	92	46.3	0.50	20	84	21.1	62.9	0.75
Valine 1	45	100.5	44.8	55.7	0.55	31	46	30.6	15.4	0.33
2	26.1	76.6	26.0	50.6	0.73	0	20	0	20	1.00
Isoleucine 1	56.5	111	57.5	53.5	0.48	25	50	25.8	24.2	0.49
2	25	56	25.8	30.2	0.55	0	19	0	19	1.00
Phenylalanine 1	62.5	164	63.5	100.5	0.62	51	130	52	78	0.40
2	24.5	98	24.5	73.5	0.75	0	18	0	18	1.00

* 1 = with glucose 2 = without glucose

Composition of the free amino acid nitrogen pool

The influence of the different amino acids given as substrates on the composition of the free amino acids pool present in the yeast cells was tested by the determination of glutamic acid, aspartic acid and alanine, which together make up about 60% of the pool amino acids. When given as substrates, valine, isoleucine and phenylalanine were also determined in the pool.

Such determinations were carried out at hourly intervals, thus yielding for each experiment a series of nine determinations from which the changes in the composition of the pool during the growth process can be followed. The results obtained showed that the changes in the pool composition followed a comparatively simple pattern and did not bring out any fundamental difference between the two yeast strains, although certain quantitative differences appeared. The composition of the major part of the amino acid nitrogen pool was in both strains fairly constant during the whole experimental period when ammonia or one of the group 1 acids were given. The amounts of amino acids found were, in the torulopsis strain (expressed as per cent of the total amino acid nitrogen content of the pool): glutamic acid 25(±3), aspatric acid 8(± 2), alanine 35(± 4). The corresponding values for the saccharomyces strain were: glutamic acid 38(± 5), aspartic acid 12(± 3) and alanine 12(± 3). The main difference between the two strains is the larger amount of alanine appearing in the pool of the torulopsis strain.

The composition of the pool was, however, significantly influenced when group 2 acids were given as substrates. During the lag period and the initial growth phase during all the first 4 hours of the experiments, the substrate amino acid (isoleucine, valine or phenylalanine) appeared not at all or only in negligible quantities in the pool. Beginning from the fourth hour, the substrate amino acid began to appear in the pool and formed, at the end of the fifth hour, 10–15% of the total amino acids in the pool. The rise of the substrate amino acid in the pool then continued steadily, until at the end of the experiment 26–30% of it consisted of the substrate amino acid. Concurrently the percentage of the glutamic acid, aspartic acid and alanine declined, until at the end of the experiments each of them formed no more than 7–8% of the pool. The conclusion can therefore be drawn that the amino acids normally occurring in large quantities in the pool are gradually replaced by the substrate (group 2) amino acid during growth. The total pool remained remarkably constant, forming about 30% of the total nitrogen content of the yeast cells in both strains.

DISCUSSION

It has been shown in the preceding paper that the amino acid, ammonia and glucose concentrations producing optimal growth are equal for both strains and independent of the nature of the substrate. According to the shape of the substrate concentration-growth curves the cell surface becomes gradually saturated with substrate molecules, which are then absorbed through the cell membrane.

The results presented in this paper have shown that for the production of a certain growth unit very different quantities of substrate are absorbed in many instances and that substrate uptake often considerably precedes growth. Both these findings could be taken as evidence for the existence of an active absorption mechanism. This was tested by a number of experiments (not reported in detail) in which it was attempted to determine the quantities of substrates present in the cell or in its surface and compare them with the substrate quantities which at the same time had disappeared from the growth medium. It was found that in every case the amounts of substrates accumulated in the cell were very much smaller than those which had disappeared from the medium. It is therefore assumed that the substrates on entering the cell are immediately metabolized or bound in such a form as not to influence the osmotic equilibrium probably existing between the cell interior and the growth medium. In the absence of any experimental evidence to the contrary, the simple assumption of a passive absorption mechanism will be adhered to, i.e. the rate of absorption of substrates determined by the concentration gradient between the cell interior and the growth medium, which in turn is influenced by the rate of metabolic processes inside the cell which cause the disappearance of the substrates.

The relation between substrate uptake and growth

Substrate uptake with relation to growth has been experimentally established and the following three different mechanisms have been found: 1) Uptake of substrate in close proportion to growth, 2) lag of substrate uptake behind growth and 3) substrate uptake in excess of growth. Proportionality of substrate uptake to growth would be expected if all the substrates taken up can be made available for the synthesis of cell constituents immediately after entering the cell interior, i.e. if none of the degradation, synthetic and possibly excretion mechanisms in the cell is slow enough to form a growth-limiting factor. This is seen to happen with the torulopsis strain, where in the presence of glucose all nitrogen-containing substrates, glucose itself, and oxygen are taken up in proportion to growth, except possibly in its very first stages. The only exception found is glutamic acid, which considerably retards glucose uptake and also represents the only case of a substrate (glucose) very significantly lagging behind growth. This can be explained by assuming that the utilization of the deamination product of glutamic acid, in all probability α-ketoglutaric acid, proceeds at a rate far exceeding that of the utilization of glucose; and that as a result growth sets in before a significant part of the glucose has time to be made available for growth.

The opposite effect, namely, the absorption of glucose in excess of growth, was observed much more frequently, and occurs in the saccharomyces strain with all N-containing substrates. In this case, glucose, which on entering the cell must immediately undergo some degradation reactions (we could not observe any accumulation of glucose within the cell), cannot, however, immediately be brought into a form suitable to enhance growth. Until this happens, additional quantities of glucose are absorbed, and as a result glucose is taken up in excess of growth. A possible mechanism explaining this finding can be sought in the results of Eaton and Klein's[9] work. These authors found that saccharomyces cells also use glucose via the initial formation of ethanol. The series of reactions leading to the formation of ethanol, possibly accompanied by its excretion and reabsorption, is time consuming and brings about the absorption of additional quantities of glucose before growth can start with ethanol as the carbon donor.

That the initial step of glucose utilization in the saccharomyces strain must be an anaerobic one is seen by the fact that only glucose is taken up in excess of growth, not oxygen, whose uptake remains strictly proportional to growth.

In the torulopsis strain such a mechanism is completely absent. The uptake of glucose in excess of growth occurs in this strain only with alanine and is here explainable by the fact that alanine contains much more nitrogen than the protein eventually synthesised in the cell and therefore causes a considerable "carbon deficit" to form, which must be equalized by the additional uptake of glucose.

Amino acid uptake which significantly exceeds growth has been observed with most amino acids in the presence of glucose in the saccharomyces strain and in both strains when glucose is absent. In these cases the dependence of growth upon the availability of the amino acid carbon skeleton is clearly apparent. The amino acids on entering the cell are immediately deaminated, and thus the concentration gradient between substrate amino acid and that in the cell is kept up and additional quantities of substrate can be absorbed from the medium. If, however, the amino acid carbon residue is but slowly utilized, this reaction represents a growth-limiting factor and growth will be slow despite the continuing absorption of amino acids from the medium. The effect is, of course, immediately abolished in the torulopsis strain upon the addition of glucose, whose carbon is readily available; in the saccharomyces strain, excess uptake of amino acids persists despite the addition of glucose, in accordance with the findings discussed above, which point to the fact that in this strain the utilization of glucose is also a slow reaction and its rate probably does not exceed that of the utilization of the amino acid carbon to a sufficient degree to dispose of the amino nitrogen liberated by the deamination of the amino acids by synthesis of protein, i.e. growth. This assumption leads to the conclusion that comparatively inefficient mechanisms for the utilization of the amino acid carbon residue (and, in the saccharomyces strain, for that of glucose, too) should cause the accumulation of unwanted quantities of N-compounds in the cell. These are excreted by the cell, as has been found experimentally (Table III), and the amount of nitrogen excreted was found as expected to be much larger when amino acids are given whose carbon skeleton is utilized incompletely.

Utilization of substrates

It has been found that the quantities of amino nitrogen absorbed for unit growth are considerably larger in the absence of glucose than in its presence. These differences, however, largely disappear when one considers that the nitrogen not actually incorporated in the cell is re-excreted into the medium. (Table III). It can therefore be assumed that the enhanced nitrogen absorption in the absence of glucose is not due to an enhanced nitrogen demand, but is due to the necessity to absorb additional quantities of carbon. This is especially apparent in the case of the group 2 acids in the absence of glucose when given to the saccharomyces strain. Here, the availability of the amino acid carbon residue is so low that no growth is possible and the carbon is utilized only to satisfy the basic energy demand of the cell, which remains therefore in a "resting state". Accordingly all the nitrogen initially absorbed in the form of the substrate amino acids is excreted, since no protein has been synthesized. The carbon utilized may not even be that of the substrate amino acid but of one of the better available ones in the pool. This appears from the fact that in the saccharomyces strain the group 2 acids tend to accumulate in the pool which, however, does not become larger owing to the simultaneous disappearance of the glutamic and aspartic acid and of alanine, which normally form its greater part.

It is also possible to evaluate the relative availability of the amino acid carbon residues by their glucose sparing action. Glutamic acid and alanine and to a lesser degree phenylalanine and isoleucine can significantly lower the glucose demand for unit growth in the torulopsis strain; the two former amino acids can also diminish glucose demand in the saccharomyces strain, whereas all others, including aspartic acid, cannot efficiently replace glucose, and even stimulate glucose demand, which can be taken as an indication that the utilization of their carbon residue not only does not generate energy but requires it, as borne out by the fact that in all cases where the presence of an amino acid in the growth medium stimulates the glucose uptake, the oxygen uptake is likewise stimulated.

Oxygen uptake as a measure of the availability of the amino acids for growth will not be discussed as such, since more useful information on possible amino acid utilization mechanisms can be gained by considering both oxygen uptake and the concurrent CO_2 production, i.e. the respiratory quotients. These are close to unity when no other carbon-containing substrate than glucose is present in the medium; when a growth medium consisting of ammonium sulphate and glucose is presented to both strains, glucose is the only material oxidized. In all cases where glucose and amino acids are given together, the respiratory quotients are lower than unity, thus indicating that the oxidation of at least part of the absorbed amino acid is an obligatory process and happens without regard to the presence of glucose. It is also apparent that the oxidation of the amino acids cannot consist in a reaction leading to the exclusive formation of the terminal oxidation products, carbon dioxide and water, since the respiratory quotients calculated when glucose and amino acids are given together are of the order of magnitude of those calculated theoretically for the total oxidation of the amino acids alone. In the absence of glucose, the respiratory quotients measured are so low as to preclude completely the presence of mechanisms oxidizing the amino acids to CO_2 and H_2O exclusively.

The oxidation of the amino acids is therefore a process whereby oxygen is taken up without the concurrent production of CO_2. Such a mechanism can consist in the reincorporation of initially formed carbon dioxide in various cell compounds, for which ample evidence has recently been brought forward by Stoppani and co-workers[10], who found that yeast cells are capable of covering a considerable part of their carbon requirements by the absorption of CO_2. It should also be remembered that the conversion of substrates like ethanol or acetaldehyde to carbohydrates can produce very low respiratory quotients, like the case demonstrated by Ingram[11], who calculates for the reaction:

$$4\ C_2H_5OH + 6\ O_2 = 2\ CO_2 + C_6H_{12}O_6 + 6\ H_2O$$

a respiratory quotient of 0.33, which is in the range of those frequently found in our experiments.

It should also be pointed out that the observation of such low respiration quotients as found by us does not support the assumption that the deamination of the amino acids in the yeast cell is mainly effected by such mechanisms as the Stickland or Ehrlich reactions, since in both reactions the amounts of CO_2 produced exceed the quantities of oxygen taken up. Thus rather high respiratory quotients would be expected.

Mechanisms of carbon utilization

While in general experiments of the type undertaken in the present work do not yield detailed information on possible mechanisms underlying carbon utilization, some significant results were obtained with respect to the role played by the carbon residue of aspartic acid. The latter, which may be fumaric acid resulting from the action of aspartase (Erkama and Virtanen[12]) or oxaloacetic acid, formed by the action of aspartic α-ketoglutaric transaminase (Bigger-Gehring[13]) is very incompletely utilized. The experimental results pointing to this fact are the following: 1) The contribution made by aspartic acid to the total oxidized material (with glucose in the torulopsis strain) is about half of that of all other amino acids, including the group 2 acids. 2) It shows no glucose-sparing action with both strains. 3) Its uptake for unit growth in the absence of glucose is in both strains much more enhanced than that of all the other group 1 acids. That all these effects are due to the comparatively low availability of the carbon residue and not to the lack of suitable deamination mechanisms is shown by the fact that in the presence of large concentrations of glucose (see the preceding paper, Table I) aspartic acid produces the best growth of all substrates, including ammonium salts. The conclusion may therefore be drawn that in both strains neither oxaloacetic acid nor fumaric acid are normal metabolic intermediates. Since both the deamination products of glutamic acid and alanine are utilized much more effectively, it may also be concluded that the reaction system leading to the formation of both fumaric and oxaloacetic acid from α-keto-glutaric acid, i.e. part of the tricarboxylic acid cycle, is not very active in the yeast cell.

Concluding remarks

All the experimental evidence presented in this work points to the fact that the main cause determining the widely different availability of amino acids for the aerobic growth of the yeast strains investigated is the ability of the cell to deal with the amino acid carbon. Investigations into the utilization of amino acids can therefore be regarded as studies concerning the metabolism of their deamination products and are of importance as such, since the yeast cell wall is in many cases impermeable to the keto acids as such and the use of amino acids can therefore be regarded as a convenient way to introduce keto acids into the normally functioning yeast cell.

A tentative scheme of amino acid utilization correlating the results presented with other reactions already known is outlined in Figure 2.

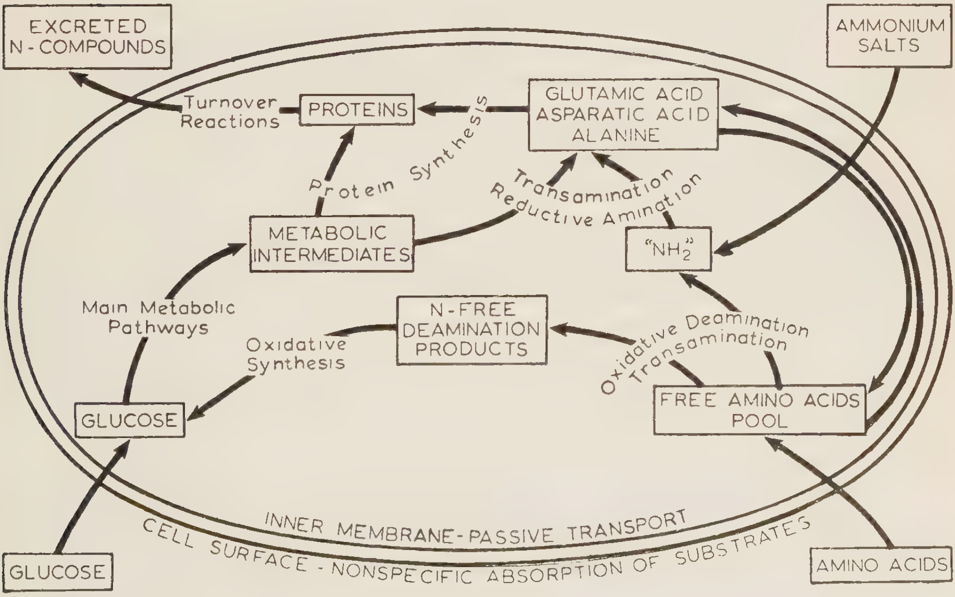


Figure 2

Tentative scheme of utilization of amino acids

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SPECTROPHOTOMETRIC DETERMINATION OF SILICA BY ITS VANADOMOLYBDATE COMPLEX

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ABSTRACT

Silica is determined spectrophotometrically by measuring the absorbence of its complex with a vanadomolybdate ion. Reproducibility of absorbence depends on adherence to a constant acidity. A comparative large excess of fluorine does not interfere. The method permits the rapid determination of silica in solutions of silicates and fluorosilicates and in insoluble silicates after alkaline fusion.

The photometric determination of phosphate by the vanadomolybdate method as modified by G. L. Bridger[2] and Gee and Deitz[4] has been used in this laboratory for several years because of its speed and its reproducibility. Because of these qualities and the relative freedom from interference, the vanadomolybdate method is now preferred to the older procedure based on the phosphomolybdate complex. The spectrophotometric determination of silica, however, is generally carried out on its yellow molybdate complex or after reduction on the blue complex[1,4]. Only the blue reduced form of the silicovanadomolybdate ion has been used so far for silica determinations[3]. In the course of our research work a rapid determination of silica in silicates and fluosilicates was required. The spectrophotometric determination of the yellow silicovanadomolybdate complex has been investigated and the method—after certain modifications—was finally adopted because of its convenience and accuracy. Compared with the phosphate complex the silicovanadomolybdate complex is rather susceptible to the acidity of the solution. However, by maintaining a strictly constant acidity, reproducible values for absorbence can be obtained. For the purpose of determination the final acidity has to be adjusted to 0.2 normal. This normality is achieved by adding reagent solution of known acidity to a neutralized solution of the sample.

As shown in Figure 1, the increase of acidity lowers the absorbence. An acidity increase from 0.2 to 0.3 N causes an absorbence decrease of approximately 80%.

As can be seen from Figure II, variations of acidity lead only to small changes in the absorbence of the phosphovanadomolybdate complex. An appreciable decrease of the absorbence is caused only by high concentrations of P_2O_5, as used for the differential spectrophotometric method.

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The presence of a large excess of fluorine—up to a molar ratio 15 F:1 SiO_2—does not affect the absorbence.

The colour should be developed at a room temperature of not less than 20°C.

PROCEDURE

Reagents

1. *Mixed reagent*

(a) 3.4 g ammonium vanadate (NH_4VO_3) in one litre of 4 N H_2SO_4.

(b) 64 g ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in one litre solution. Equal parts of solutions (a) and (b) are mixed in the quantity required.

2. *Standard silica solution*

(a) Solution A: Five grams of sodium silicate are dissolved in 200 ml hot water, 1–2 pellets of sodium hydroxide are added. The solution is left for one day, filtered, diluted to one litre and stored in a plastic bottle. The silica content is determined gravimetrically.

(b) Solution B: Five ml of solution A are diluted to one litre. 1 ml is equivalent to 100–150 $\mu g\, SiO_2$.

3. 0.2 N NaOH—to be kept in a plastic bottle.

4. 0.2 N H_2SO_4.

Remark: All reagents, especially sodium hydroxide and alkali carbonates used for fusion, must be silica-free.

Apparatus: Beckman DU spectrophotometer.

Determination of silica in alkali silicate.

A known quantity of the silicate is dissolved in water and diluted in a volumetric flask up to the mark. Aliquots containing 0.5–1.0 mg silica are placed into a volumetric flask of 100 ml and neutralized to phenolphthalein with 0.2 N sulphuric acid, 10 ml mixed reagent are added and the flask is filled up to the mark. In the same way a blank and two standards containing respectively 0.5 mg and 1.0 mg silica are prepared.

The colour is developed at a room temperature of not less than 20°C and measured after thirty minutes.

Corex cells of 1.00 cm are used and the absorbence measured at 390 $m\mu$. The appropriate filter for the 320–400 $m\mu$ range must be used.

Conversion of absorbence measurements into mg SiO_2, as shown in Table I, are based on curve D in Figure 1.

Calculation: The content of SiO_2 in mg in the aliquot can either be read from the graph or calculated:

$$SiO_2\text{, mg in aliquot} = 2.18 \times \text{absorbence.}$$

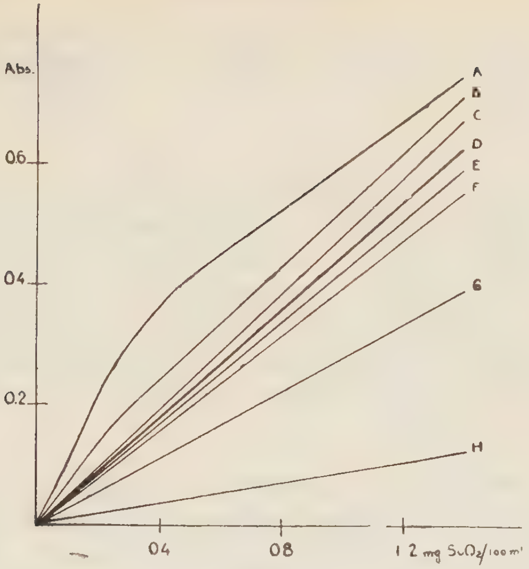

Figure 1
Absorbence vs content of SiO_2 in mg
Influence of varying acidity on SiO_2-determination

A: 0.10 N H_2SO_4
B: 0.15 N H_2SO_4
C: 0.175 N H_2SO_4
D: 0.20 N H_2SO_4
E: 0.213 N H_2SO_4
F: 0.225 N H_2SO_4
G: 0.25 N H_2SO_4
H: 0.30 N H_2SO_4

Remarks

1. All measurements indicated in this paper were carried out with the Beckman DU spectrophotometer.

2. The factor 2.18—tangent in the graph "absorbence versus concentration"—can vary within narrow limits, depending on small differences in the composition of the mixed reagent.

Table I shows the spectrophotometric results as compared with those obtained gravimetrically.

TABLE I
Determination of silica in sodium silicate

Exp. No.	Taken SiO_2 (mg)	Absorbence at 390 m	Found SiO_2 (mg)
1	0.268	0.123	0.269
2	0.536	0.243	0.530
3	0.670	0.304	0.670
4	0.804	0.367	0.800
5	0.938	0.431	0.940
6	1.072	0.495	1.078
7	1.206	0.555	1.210
8	1.340	0.615	1.340

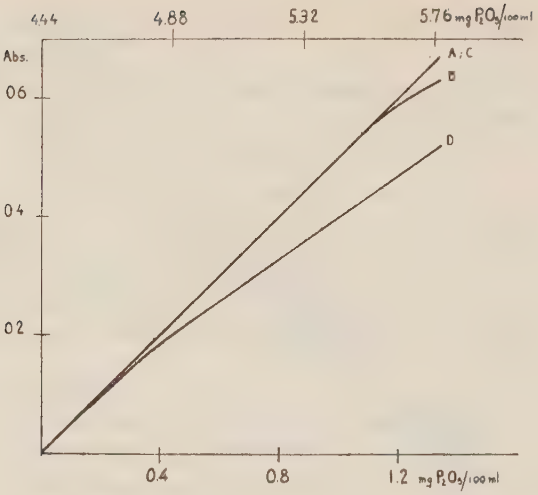

Figure 2

Influence of varying acidity on P_2O_5-determination

The concentration for non-differential measurements are stated on the lower abscissa, those for the differential one are given on the upper abscissa.

A: 0.2 N H_2SO_4, non-differential
B: 0.6 N H_2SO_4, non-differential
C: 0.2 N H_2SO_4, differential (curve C identical with curve A)
D: 0.6 N H_2SO_4, differential

Interference of fluoride

The extent of fluorine interference with the spectrophotometric determination of silica was investigated by adding increasing amounts of sodium fluoride to a measured aliquot of a sodium silicate solution.

As evident from Table II, a molar ratio of 15 F:1 SiO_2 can still be tolerated without appreciably affecting the absorbence.

TABLE II

Fluorine interference

Exp. No.	Taken SiO_2 (mg)	F added (mg)	Mol ratio F/SiO_2	Absorbence at 390 mμ	SiO_2 found (mg)
1	0.670	–	–	0.306	0.667
2	0.670	0.67	3.2	0.308	0.672
3	0.670	1.68	7.5	0.305	0.665
4	0.670	3.35	15.8	0.300	0.654
5	0.670	3.90	19.2	0.295	0.644
6	0.670	7.90	38.5	0.306	0.667
7	0.670	15.8	75.0	0.260	0.567
8	0.670	16.7	78.5	0.078	0.170

In order to determine silica in the presence of larger amounts of fluorine, e.g. in soluble fluorides, it is preferable to convert the silica to potassium fluorosilicate with subsequent titration[5].

Interference of phosphate

The interference of phosphate with the spectrophotometric determination does not come within the scope of this paper. However, as seen from curves in Figure 1 and Figure 2, it is possible to determine phosphate in the presence of silica using a higher acidity[2], which eliminates the absorbence effect of the silica. The total of phosphate and silica can be obtained under the conditions outlined in the procedure. From the difference of the results found, the silica content can be calculated.

Determination of silica in insoluble silicates

100 mg of a kaolin sample of local origin, ground to 200 mesh, was fused in a platinum crucible using 2–3 g of a mixture of sodium and potassium carbonate (8 + 1).

The melt was dissolved in water and hydrochloric acid added to an acid reaction. The solution was transferred into a 250 ml volumetric flask and filled up to the mark. On an aliquot of the solution containing 0.5–1.0 mg SiO_2 (about 5 ml), the spectrophotometric determination was carried out as described above.

The content of silica in 0.5 g of kaolin was determined gravimetrically. The comparision of the results is given in Table III.

TABLE III

SiO_2-*determination in kaolin*

Exp. No.	Weight (g/250 ml)	Aliquot (ml)	Absorbence	SiO_2 (mg)	Found %	Δ %
1	0.1016	5.0	0.375	0.818	40.3	+1.0
2	0.1047	5.0	0.390	0.850	40.6	+1.75
3	0.1034	5.0	0.375	0.818	39.5	−1.0
4	0.1029	5.0	0.375	0.818	39.8	−0.25

Note

1. The fusion was started with a Bunsen burner and completed by heating the crucible in a muffle at 1000°C for half an hour. Only 2–3 mg remained insoluble when the melt was dissolved. As apparent from the analysis in Table 3, this small percentage of insolubles has no influence on the results.

2. In case minor elements have to be determined, the residue should be filtered, ashed and fused with potassium bisulphate and the solution added to the main filtrate.

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THE USE OF THERMISTORS IN THE FLOW-METHOD FOR MEASURING RAPID REACTIONS: THE DEHYDRATION OF CARBONIC ACID AS A TEST-REACTION

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ABSTRACT

A continuous flow apparatus for the measurement of rapid reactions is described. The thermal method is employed, the innovation consisting in the use of thermistors as temperature indicators. Performance data of the apparatus are given. The suitability of the dehydration of carbonic acid as a test-reaction for this kind of apparatus and the possibility of measuring the rate of evolution of carbon dioxide are discussed.

INTRODUCTION

In the continuous flow-method of Hartridge and Roughton[1] for measuring rapid reactions the two reagent solutions are brought together in a suitable mixing chamber, and the mixture is then driven through an observation tube where some physical property which indicates the progress of the reaction is measured at a certain distance from the mixing chamber. From this distance, together with the known rate of flow, the time during which the two reagents had been in contact at the point of measurement can be calculated, and conclusions concerning the rate and mechanism of the reaction can be drawn in the usual way. In order to obtain values for the extent of the reaction at different times, it is customary to carry out measurements at different rates of flow; alternatively, the physical property concerned can be measured at different points along the observation tube. Changes in electric conductivity, *p*H, light absorption and temperature have been used for this purpose. Only the latter two are useful for a general purpose apparatus where the reagent solutions contain a buffer or other foreign electrolyte. For our apparatus, which is primarily intended for the investigation of the hydrolysis of bromine and chlorine, we chose the thermal method[1], using thermistors as indicators. As far as we are aware, these have not been used for the purpose before, in spite of a suggestion by Roughton[1] some years ago.

The measuring devices necessary for satisfactory performance are much simpler in this case than in the case of thermocouples.

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DESCRIPTION OF APPARATUS

The reagents were contained in 10-litre Pyrex flasks with ground-glass connections and surrounded by wire netting. A pressure of up to about 700 mm was applied over the solutions from a nitrogen cylinder. When desirable, the driving pressure along the observation tube could be increased up to about 1300 mm by applying a vacuum at the exit. This device avoids large excess pressure which might endanger the glass vessels used. The mixing chamber was an ordinary 3-way capillary T-stopcock, the stem acting as observation tube. The inner diameter was 1.606 ± 0.006 mm, calculated from the length of a weighed quantity of mercury in the observation tube, and was homogeneous to $\pm 1\%$. This was ascertained by observing with a cathetometer the length of a small drop of mercury in different parts of the capillary. The thermistors (Stantel, type F2311/300) were inserted through holes bored into the capillary and held in place by picein. The capillary observation tube was followed by two comparatively large volumes (0.8 and 5.0 cc respectively) which were interposed between the last three thermistors, thus enabling reaction times up to about 1 sec to be measured. Eight thermistors were employed in all. The mixing chamber, observation tube and thermistors were encased in a block of pure solid paraffin (m.p. 60°C) which was cast around them in order to increase thermal insulation and decrease the danger of breakage. All the stopcocks, connections, etc., were suitably secured to withstand the excess pressure, and the reaction vessels were connected to heavy iron plates in order to prevent them from floating as they become empty. All the parts described above were immersed in the thermostat. The thermostat was heated by a single 500 W immersion heater in series with two resistances. One of them was regulated by hand, according to room temperature, to keep the temperature very slightly below that desired; the other could be short-circuited by a Sunvic hot-wire electronic relay (type EA3 (M)), controlled by a mercury thermoregulator, having a capillary of 0.4 mm diameter. Using a large double stirrer, we found that with this arrangement temperature fluctuations were approximately ± 0.002°C inside the thermostat when measured with a Beckmann thermometer, and presumably less in the vessels containing the reactants.

The thermistors were calibrated with the aid of a Beckmann thermometer (subdivided to $2 \cdot 10^{-3}$°C).

The dependence of the resistance R on temperature[2] is given by

$$d \ln R/dT = -B/T^2.$$

At 25 and 12°C, we found B approximately equal to $3 \cdot 10^3$ deg and almost independent of temperature. However, since during each experiment the temperature range is only a few tenths of a degree, we treated the quantity B/T^2 as a constant. At $25 \pm 0.5°$, its value was approximately $3 \cdot 10^{-2}$ deg^{-1}, whereas R lay between 1400 and 1800 ohm. Resistances were measured with a 5-decade Rubicon "High Precision" Wheatstone Bridge and a mirror galvanometer (Kipp and Zonen, double coil, Model A 53) whose sensitivity is given as 1 mm deflection for 0.1 to 1 × 10^{-9} A, at a distance of 1 m. With a current of approximately 40 μA through each thermistor, an unbalance of 0.1 ohm causes a deflection of about 1 mm on the galvanometer scale. It follows that temperature differences can be estimated with an accuracy of ± 0.001°C. A total temperature difference of 0.1°C can thus be measured with an accuracy of 1%.

The Beckmann thermometer was calibrated against an ordinary thermometer with 0.1°C subdivisions. The *absolute* value of temperature measured by the thermistors was therefore accurate to at most 0.1°, but was probably much less, because of slight changes of R. For our purpose it was important that these changes leave B unaffected. This was found to be the case, and for one particular thermistor, B was re-measured after a period of more than one year; the change found was within the limits of experimental error.

PERFORMANCE TESTS

1. *Efficiency of mixing and adiabaticity*

A 0.121 M solution of NaOH was mixed with a 0.0457 M solution of HCl. The formation of water from its ions is "instantaneous" in comparison with the rate of reactions which can be measured in our apparatus[1,3]. The "rate of reaction" measured in this case gives the rate of mixing. At flow-velocities between 150 and 220 cm/sec, we found that within the limits of experimental error, mixing was complete at the first thermistor. The amount of acid neutralized was calculated from the known concentration of the reactants and the concentration of NaOH in the mixture. From this, together with the heat of neutralization[4] ($\Delta H = 13.631$ Kcal/mol) we calculated the expected rise in temperature. As a mean of three experiments, the measured value was 0.96 ± 0.02 of the theoretical value, which was considered satisfactory.

2. *Testing of the apparatus using a known reaction*

We re-measured the reaction between potassium bichromate and sodium hydroxide, a reaction which proceeds in steps:

$$\mathrm{Cr_2O_7^{=} + H_2O \rightarrow 2\,HCrO_4^{-}} \qquad \text{(measurable)} \qquad (1)$$

$$\left.\begin{array}{r}\mathrm{HCrO_4^{-} \rightarrow H^{+} + CrO_4^{=}}\\ \mathrm{H^{+} + OH^{-} \rightarrow H_2O}\end{array}\right\} \qquad \text{("instantaneous")} \qquad (2)$$

We duplicated exactly the conditions under which La Mer and Read[5] had carried out their measurements and obtained $k = 9.4$ sec^{-1} at 25°C, in reasonable agreement with the previous authors' value of $k = 8.83 \pm 0.41$ at the same temperature. Our graph of ΔT vs. time is shown in Figure 1 and of $\log (\Delta T_\infty - \Delta T_0)/(\Delta T_\infty - \Delta T_t)$ vs time in Figure 2. The initial "instantaneous" rise in temperature is due to the ionization, according to (2), of that part of the $HCrO_4^-$ which is present in the original solution, in equilibrium with $Cr_2O_7^{=}$.

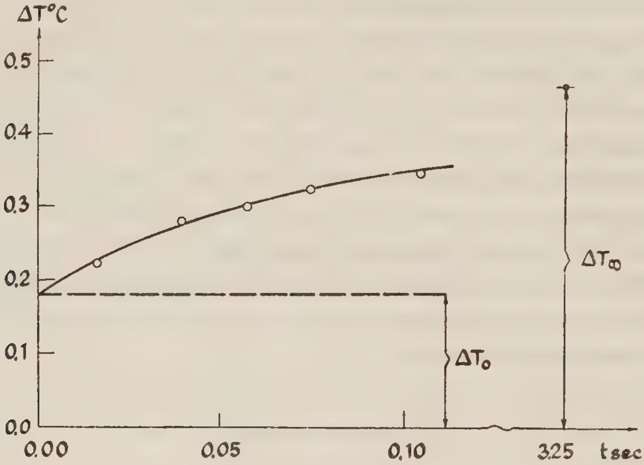

Figure 1

The rise of temperature, ΔT, as a function of time (in seconds) for the reaction between potassium bichromate and sodium hydroxide

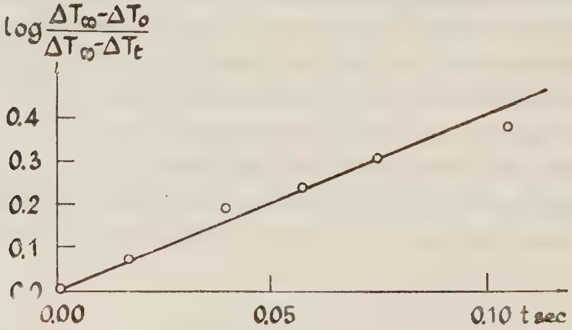

Figure 2.

log $(\Delta T_\infty - \Delta T_o) / (\Delta T_\infty - \Delta T_t)$ vs time, for the reaction between potassium bichromate and sodium hydroxide

THE DEHYDRATION OF CARBONIC ACID

The reaction

$$NaHCO_3 + HCl \rightarrow CO_2 + NaCl + H_2O$$

has been investigated by Roughton[1,6] and found to proceed in two stages:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \qquad \text{("instantaneous")} \qquad (3)$$

$$H_2CO_3 \rightarrow CO_2 + H_2O \qquad \text{(measurable)} \qquad (4)$$

We intended to use this reaction as a test for our apparatus, with 0.41 M $NaHCO_3$ and 0.38 M HCl. However, using various observation tubes, we obtained results which were not reproducible. In a preliminary observation tube having 4 thermistors and a diameter of 1.52 mm, we obtained $k = 27.1 \pm 1.0$ sec^{-1} as a mean of two experiments, where the reaction was observed up to about 50%. The agreement with the value of 28.0 sec^{-1} calculated from Roughton's data is satisfactory, although our experimental points are rather few.

In another preliminary observation tube having 8 thermistors, thus enabling longer times to be measured, and a diameter of 2.0 mm, we obtained temperature rise vs time curves in which three portions could be distinguished. The instantaneous temperature rise corresponding to reaction (3) was followed first by the slower rise due to reaction (4), and then by an approximately exponential decrease corresponding to a third, endothermal step. In three experiments carried out at different rates of flow, this endothermal reaction started at different times, but *always* just after the fifth thermistor. In the observation tube described in the first part of this paper similar results were obtained. However, the endothermal step set in much sooner and the highest temperature obtained was considerably lower than the value of ΔT_∞ expected after reactions (3) and (4) had gone to completion (calculated from[7] $\Delta H = -2.248$ Kcal mole^{-1}). We did not attempt to calculate the rate constant of reaction (4) from these experiments.

We propose that the endothermal reaction responsible for the decrease in temperature is the evolution of CO_2. From solubility data of CO_2 at various temperatures[7] we calculate $\Delta H = 4.70$ Kcal mole^{-1} for this reaction. From this, it is possible to calculate the value of $-\Delta T_\infty$ expected on the basis of the assumption that the solution leaving the observation tube is 0.031 M in CO_2, i.e. saturated[7] at our atmospheric pressure (690 mm). We found that this temperature is not quite reached, i.e. the liberation of CO_2 is incomplete after 1.1 to 1.5 sec.

From the lack of reproducibility of our results, and from the fact that the break in the curve sets in not always at the same extent of reaction, but always at the same *point* in a given observation tube, it can be concluded that the solution becomes supersaturated and that the evolution of gas is initiated by some slight inhomogeneity in the observation tube. It should therefore be possible to delay the start of the gas evolution, so that its rate can be measured after reaction (4) has gone to completion, and its kinetics investigated*. However the quantitative interpretation of the result would be very difficult, because of the pressure drop along the observation tube.

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* The kinetics of CO_2-evolution and of the reverse reaction, the uptake of CO_2 by water, have been investigated manometrically by Roughton *et al.* (Roughton, F.J.W., 1941, *J. Biol. Chem.* **141**, 129; Roughton, F.J.W. and Booth, V.H., 1946, *Biochem. J.*, **40**, 309) under conditions which are very different from those obtaining in our observation tube. The assumption that diffusion of CO_2 through a stationary liquid layer is the rate-determining step has been shown to be justified

In any case it can be concluded from our experiments that the *dehydration of carbonic acid is not a suitable test-reaction when more than about* 0.03 *M carbonic acid is formed.*

The authors are indebted to Prof. G. Stein for his continued interest in their work. Two of the authors (E.G. and B.P.-H.) wish to thank the Research Council of Israel for supporting part of this investigation.

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SOME ASPECTS OF THE SPECTROCHEMICAL DETERMINATION OF URANIUM IN SILICATE MINERALS RICH IN OTHER RARE ELEMENTS

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ABSTRACT

The specific behaviour of the uranium spectrum obtained from a silicate rock rich in other rare elements in the presence of GeO_2 and other enhancing agents was investigated. Uranium was determined in rock containing allanite and other minerals by the "method of single adding" using GeO_2 as an enhancing agent. The results obtained are in good agreement with the chemical determination.

INTRODUCTION

In spite of the existence of many different methods for the determination of uranium in various rocks and ores, a spectrochemical analysis is very desirable, especially if it can be carried out without first treating the samples chemically. The usefulness of spectrochemical analysis of uranium is limited by the relatively low sensitivity of detection. The determination of uranium, even in concentrations of 0.1%, is difficult, especially in the case where the investigated mineral is rich in some other rare elements, for example Th and Ce, which give rise to a very strong background.

In order to increase the sensitivity of detection of uranium, Strasheim[4] used an enhancing agent. He determined the suitability for this purpose of SnO, CdO, BiOCl, RbCl, $PbCl_2$, NH_4Cl, Sb_2O_4, SbOCl, Tl_2O_3, TeO_2 and HgO and showed that the greatest enhancement is given by $PbCl_2$, SnO and HgO. It was found that NH_4Cl, RbCl and TeO_2 were unsuitable. Strasheim used $PbCl_2$. He presumed that its function was more that of a background suppressing agent than that of an enhancing agent of the uranium spectrum. The samples were mixed with an equal quantity of $PbCl_2$ and burned to completion in 90 seconds.

In the work of Burgener, Gurney and Rudnik[1] the enhancement of the uranium spectrum is achieved by the use of lead and palladium chlorides.

Ivanov[7] studied the influence of Al, Si, Fe, Ti, Mn, Pb, Cu and others on the uranium spectrum.

Tonani, Cocco and Serrini[6] have examined the effect of CuO, Cu_2O, PbO and particularly $PbCl_2$ as enhancing agents. By comparing the effect of $PbCl_2$ on uranium and on zirconium, using the moving plate technique, they concluded that lead chloride affects only the behaviour of the uranium volatilization, and its action

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is specific. In their work, the sample was pre-arced for 1 minute and the exposure was 2 min. 30 sec.

The present work sought initially to investigate other enhancing agents for silicate minerals rich in other rare elements. It was decided to try such materials which were volatile. Accordingly, the suitability of CuO, Ag_2SO_4, ZnO, CdO, HgO, Ga_2O_3, Tl_2O_3, GeO_2, SnO (and SnO_2), $PbCl_2$ (and PbO), As_2O_3, Sb_2O_5, Bi_2O_3 and Li_2CO_3 was investigated and all, with the exception of Li_2CO_3, were found to be more or less effective.

The progress of the emission from samples was followed with moving plates and it was found that the effect of the enhancing agent was both to enhance the uranium spectrum and suppress those of the refractory elements. In this way it was clearly established that the enhancing agent behaves specifically for uranium.

The rock examined was a complicated mixture of allanite (a thorium rare earth silicate mineral), hematite, quartz, feldspar and fluorite. It also contained a considerable quantity of niobium. If this rock is analysed under commonly used conditions, i.e. by mixing it with graphite powder, there results such a strong background that the sensitive lines of uranium are obscured.

A 15 mg sample was mixed with the enhancing agent in equal amounts and the spectrum studied with moving plates. When the sample started to burn, the background was suppressed and the intensity of uranium lines was increased. At the same time, the lines of Ca, Sr, Ba, Al, Sc, Y, La, Ce, Nd, Zr, and Th were suppressed. Calcium was so depressed during the early period of arcing that no CaF bands were recorded.

Later on a rapid increase of background intensity was observed as well as of the lines of those elements suppressed at first. The uranium spectrum became either very weak or not visible at all. The moment at which the enhancing agent was completely evaporated and the strong background emitted was observed by a change in brightness of the arc.

The time it took for a strong background to appear was different for the various enhancing agents. For example, with very volatile agents (CdO, HgO and As_2O_3), the strong background appeared after a few seconds of burning; with $PbCl_2$ and ZnO, after 15 seconds; with GeO_2, after 45 seconds; and with SnO, after 75 seconds.

The effects described are shown in Figure 1, using GeO_2 as an example.

Strasheim reported unsatisfactory accuracy in determining uranium, using La as the internal standard, The reason for this is probably the different behaviour of U and La in the mixture of the sample and enhancing agent (Figure 1).

The influence of GeO_2 on the uranium spectrum was also investigated in the mineral thorite. The change of intensity of the lines of various elements during the arcing of thorite with GeO_2 (Figure 2) also indicates the specific behaviour of the uranium spectrum.

The observed effects of enhancement of the uranium spectrum and suppression of the spectra of other refractory elements may be interpreted as due to the influence of the enhancing agent on the respective vapour pressures. Such a suggestion has been

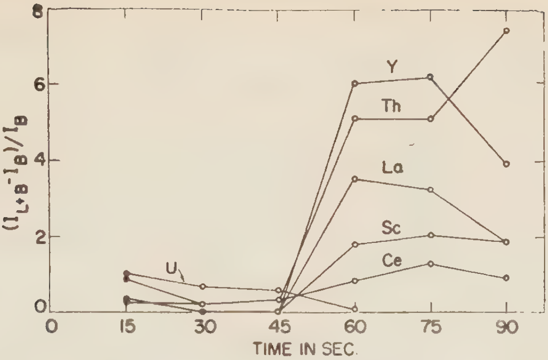

Figure 1

The change of intensity of various refractory elements during the arcing of a sample rock containing allanite mixed with GeO_2

Analysis lines:	U	4244.37 A	La	4333.73 A
	Sc	4246.83 A	Ce	4222.60 A
	Y	3982.60 A	Th	4019.14 A

I_L is the intensity of the analysis line

I_B is the intensity of the background

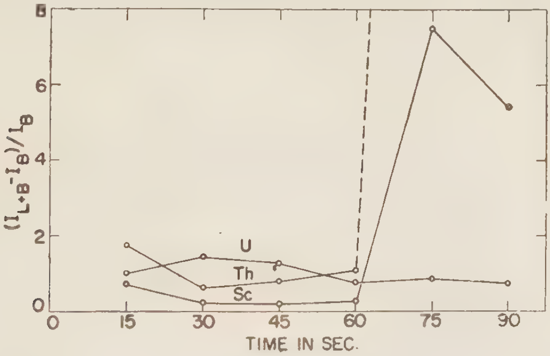

Figure 2

The change of intensity of U 4244.37 A, Sc 4020.40 A, and Th 4019.14 A during the arcing of a thorite sample mixed with GeO_2

I_L is the intensity of the analysis line

I_B is the intensity of the background

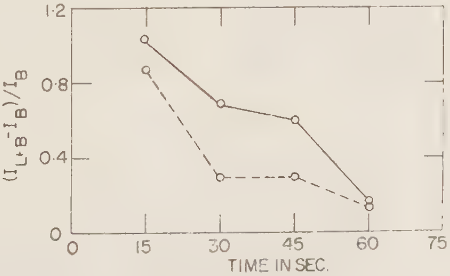

Figure 3

The intensity ratio of uranium analysis line U 4244.37A to background

———— The sample mixed with GeO_2

– – – – The sample mixed with $PbCl_2$

I_L is the intensity of the analysis line, and

I_B is the intensity of the background given by Shvangiradze[3], who made observations on the spectra of Sm and other rare earths in the mixture of rare earths and graphite.

DETERMINATION OF URANIUM IN A ROCK CONTAINING ALLANITE

As can be seen from Figure 1 it is best to determine uranium using an exposure in the early part of the burning (first 20 sec) before the appearance of a strong background and the spectra of other refractory elements.

GeO_2 seems to be the most suitable enhancing agent, because with it the intensity ratio of the uranium analysis lines to background is greatest. Moreover, the relatively long time of arcing of the sample before a strong background appears allows greater reproducibility of spectra. (Figure 3).

The determination was made by the "method of single adding" adapted to semi-quantitative determinations[5]. The method of addition in general is especially important n the case of rocks and minerals of very complicated compositions, when the intensity of the analysis lines is extremely sensitive to compositional changes.

Uranium in the quantity of 0.25% was added to the sample in the form of U_3O_8.

Working conditions are given in Table I.

TABLE I

Working conditions

Spectrograph	Hilger Automatic, No. E 478. Glass optics
Wavelength range	3860–5500 A
Analysis line	U 4244.37 A
Mode of illumination	The image of the light source was projected onto the collimator lens
Slit width	0.015 mm
Light source	D.C. arc, 10 amp
Electrode gap	6 mm
Electrodes	United spectro-tech. graphite. Upper electrode of 4.5 mm diameter with a rounded end. Lower electrode of 6 mm diameter, 3 mm deep, a wall thickness of 0.7 mm
Samples	Finely ground samples of 15 mg mixed with 15 mg GeO_2
Exposure time	20 seconds
Photographic plates	Kodak spectroscopic plates II-F
Processing	Developed at 20°C for 3 min in D–19. Fixed in Kodak Acid Fixer. Development stopped in acetic rinse bath

The analysis line U 3932.03 A could also be used in this work, because the line Ca 3933.7 A, which in general masks this line of uranium, is very much suppressed. The U 3932.03 A line is suitable when the concentration of uranium is high. The U 4341.69 A line used by Ivanov[7] and also by Tonani, Cocco and Serrini[6] is suitable for low concentrations of uranium. In the first initial stages of burning no coincidence of other spectral lines was observed. The U 4241.67 A line used by many authors[4,7,8] cannot be used when zirconium is present in great quantities.

TABLE II

Determination of uranium by "method of single adding"

Plate No.	I_x	$\overline{I}_x$	I_{x+z}	$\overline{I}_{x+z}$	$I' = \frac{\overline{I}_{x+z}}{\overline{I}_x}$	$V_1 = \frac{\overline{s}_1 \cdot 100}{\overline{I}_x}$	$V_2 = \frac{\overline{s}_1 \cdot 100}{\overline{I}_{x+z}}$	$\overline{V} = \frac{V_1 + V_2}{2}$	X (b=1)	X_{min} (b=0.8)	X_{max} (b=1.2)	Chemical determination*
1	1.14		2.24									
	1.29		2.17									
2	1.40		2.48									
	1.63		2.88									
3	1.14		1.88									
	1.34		2.55									
		1.30		2.35	1.8	3.3%	4.2%	3.7%	0.31%	0.21%	0.45%	0.34%+0.01
4	1.35		2.10									
	1.30		2.80									
	1.29		2.24									
5	1.19		2.39									
	1.09		1.78									
	1.39		2.70									

x — concentration of uranium

I_x and I_{x+z} — the ratios of intensity of U 4244.37 A corrected for background to the intensity of the background in the sample respectively without and with the addition of 0.25% uranium

$\overline{I}_x$ and $\overline{I}_{x+z}$ — the corresponding average values

$$\overline{s}_1 = \sqrt{\frac{\Sigma(I_x - \overline{I}_x)^2}{n(n-1)}}$$

$$\overline{s}_2 = \sqrt{\frac{\Sigma(I_{x+z} - \overline{I}_{x+z})^2}{n(n-1)}}$$

* The chemical determinations were made by volumetric and fluorometric methods in the Analytical Laboratory of the Israel Atomic Energy Commission. The indicated error represents the 95% confidence interval based on 8 independent measurements on the sample.

In this work the background on the short wavelength side of U 4244.37 A was used as an internal standard.

Photometric measurements were made with a Hilger non-recording microphotometer. The photographic emulsion was calibrated with a 7-step filter in the region of the analysis line of uranium. The intensity of the line was corrected for background.

The unknown concentration x of uranium was calculated according to the "method of single adding"[5]. The results are set out in Table II.

The intensity I of a line is related to the concentration C by the equation

$$I = aC^b$$

where b is normally[2] between the limits 1.0 ± 0.2, and $x_{\max}$ and $x_{\min}$ in Table II are computed using these extreme values.

The results obtained are in good agreement with chemical determinations.

The applicability of this method using GeO_2 for various other minerals seems worth further investigation.

This work was carried out in the Spectroscopic Laboratory of the Weizmann Institute of Science, Rehovot.

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ISRAEL CHEMICAL SOCIETY
SECTION OF ANALYTICAL CHEMISTRY
ANNUAL MEETING,
HAIFA, MARCH 10–11, 1959

The annual meeting of the Section of Analytical Chemistry of the Israel Chemical Society, held at Haifa on the 10th and 11th of March, 1959, was devoted to problems of analytical chemistry in industry, and was distinguished by the participation of Prof. I. M. Kolthoff of the University of Minnesota. There was a record attendance of well over two hundred members.

The inaugural session was opened by the chairman of the section, Dr. M. Beer-El, after which Prof. I. M. Kolthoff delivered a short lecture on "The Importance of Analytical Chemistry", given in full at the end of this report.

A lecture on "Salt Water Purification" by Prof. K. Spiegler was read by Prof. D. Ginsburg in Prof. Spiegler's absence due to illness. The various methods of water purification were discussed from an economic point of view. In Prof. Spiegler's opinion the old-fashioned distillation method, improved by the use of a multistage arrangement, is in many cases the most profitable method even today. A cheap process for the sweetening of sea water for irrigation has not yet been developed.

Dr. S. Davidson described analytical control at Fertilizers and Chemicals Ltd., Haifa, producers of basic chemicals and a whole range of nitrogen, phosphate and potassium fertilizers. The works comprise a sulphuric acid plant, a superphosphate plant, and an ammonia and ammonium sulphate plant. Besides this, potassium sulphate, dicalcium phosphate, nitric acid, phosphoric acid and sodium phosphates, e.g. sodium tripolyphosphate and sodium tetrapyrophosphate, are produced.

Special emphasis was given in the lecture to the operation of a plant like the ammonia plant and to its chemical control, which is probably less known.

The methods used in this plant comprise usual analytical methods and special instrumental analysis: For the determination of CO and CO_2 in the parts per million range the electro-conductivity method is used; for the determination of argon, ammonia and hydrogen the thermoconductivity method is used.

The problem of the organisation of chemical control in a combine comprising 12–14 major plants was discussed and work distribution between a control laboratory and laboratories situated in the plant area was described. Test schedules for each plant which have been worked out include analyses to be done, their frequency and required man hours. These provide the basis for the laboratory costs which are charged to each plant. Laboratory manuals for each plant have been issued. They contain all testing methods and procedures which are required for the chemical control of the specific plant.

Consideration is being devoted to the use of classical analytical methods and internationally recognized standard methods in relationship to quick methods and instrumental analysis. The trend is to use these latter methods for plant control and to analyse outgoing products by standard methods which are accepted by the customer.

Mr. S. Harel drew a picture of the work of the analytical research group at Fertilizers and Chemicals Ltd. Examples discussed were:

1. The differential spectrophotometric method for P_2O_5 determination by the phosphovanadomolybdate complex;
2. The application of a similar complex for SiO_2 determination;
3. The oxalate method for rapid determination of water-soluble P_2O_5 in superphosphate;
4. Fluorine determination a) gravimetrically as PbClF, b) volumetrically by thorium nitrate, c) spectrophotometrically with alizarin sulphonate or thoron;
5. Introduction of analytical methods of specific importance for the superphosphate industry: a) the acid requirement determination, b) the discrimination between moisture and water of crystallization;
6. The use of dithiozone;
7. Determination of potassium in fertilizers by tetraphenylboron and flame photometer:
8. The determination of organic sulphate, sulphonate and carboxylate in technical detergent-mixtures.

Dr. Alon attempted to clarify some misconceptions regarding the place of analytical chemistry in chemical research, using as an example the laboratories of the Israel Mining Industries, Haifa. He described the facilities, organisation and work of the analytical department of the Company, The coordination between research and analytical team was considered, with some examples of how analytical work is an integral part of the research project.

The analytical chemist's role, approach and decision that leads to the development, application and adaptation of a certain method were illustrated by examples and outlines connected with various research projects of Israel Mining Industries, Haifa, by Engineer B. **Bernas**. The interdependent use of ion exchange, chelation and complexometric titration in the determination of sulphites, dithionates and sulphate ions in the presence of each other* was chosen as an example of selecting the needed analytical tools to solve a given problem.

Discussed in brief were the following:

1. The uses of specific reagents as exemplified by the spectrophotometric determination of aluminium in a variety of complex materials as the Eriochrome Cyanine-R complex.
2. Solvent extraction techniques using selective organic reagents at selected *p*H conditions for the micro-determination of metals were cited; as an example the determination of iron as iron cupferrate (in concentration range of 10–50 γ Fe/aliquot) in electrolytic manganese was presented.
3. The preferential application of flame photometric methods over time-consuming conventional procedures as applied to the determination of Ca and Mg in minerals and ores. Tables of comparative results were given.
4. Industrial application of TBE as a heavy liquid medium for the up-grading of ores being developed at Israel Mining Industries' mineral dressing laboratories required a procedure for the micro-determination of residual TBE on ores. The development of a method was outlined.**

Dr. I. Schnerb described the processes employed in the production of potassium chloride at the Dead Sea Works, Ltd. The analytical chemist must supervise and analyse the materials at all stages, beginning with frequent analyses of the Dead Sea waters and of the solutions in the evaporating pans where sodium chloride and carnallite precipitate, the separation of the silvanite, the flotation of the potassium chloride and even the analyses of the samples being loaded on the ships at Haifa. The responsibility of the analysts in sampling and deciding on the appropriate method of analysis was emphasized.

It was interesting to note that there were constant changes in the concentrations of the various salts in the Dead Sea, and the specific gravity of the sea water at Sodom has risen from 1.190 to 1.205 during the last two years. In the pans, sodium chloride precipitates first until a sp. gr. of 1.300 is attained. At a sp. gr. of 1.350, almost all the carnallite is precipitated. The mother liquor, which contains 12 g of bromine per litre as compared with 4 g in the sea water, is exploited for the production of bromine.

Potash sold in the world market is estimated in per cent of K_2O. The main product of the Dead Sea Works is 61% K_2O, corresponding to 96.5% KCl. Another grade is 98% KCl, when very strict requirements with respect to the presence of magnesium in the product are met.

The standard methods for estimating potassium are the perchlorate and the chloroplatinate methods, which are of equal accuracy. The flame photometric method is very useful, but it cannot be employed on the shores of the Dead Sea because of the high concentration of potassium salts in the air. In general, accepted methods of analysis have often to be adapted to the special conditions and requirements existing at the Dead Sea and in the salt solutions to be analysed. In the laboratories of the Company, good use has been made of a method using cobaltinitrite in which potassium is estimated by measuring the volume of the precipitate.

The analytical chemist of the Dead Sea Works plays an important part in the research work conducted in order to improve the processes of production. This often requires very exact phase studies. The minerals and the water sources of the surroundings of the Dead Sea are also being investigated. Two years ago a spring was discovered in which the concentration of potassium chloride was five times as great as that of the sea water. A deposit containing 8 per cent potassium chloride was found.

The analytical chemist of the Dead Sea Works, sometimes in cooperation with others, has succeeded in surmounting many difficult analytical problems connected, sometimes not straightforwardly, with the present production of the company. Of these, the discovery of rubidium and lithium in the Dead Sea waters and plans for their separation were mentioned.

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* Soffer, N. and Alon, A., in preparation.

** Alon, A., Ader, D. and Bernas, B., in preparation.

An interesting analytical problem solved was that of the chloride–bromide ratio in plants and in animals, including human beings.

The importance of analytical chemistry. I. M. Kolthoff, *University of Minnesota* (*U.S.A.*). In the early days of the development of chemistry as a science, the main emphasis was on the *detection* of inorganic constituents and classes of compounds like acids and bases. The classical hydrogen sulphide system for the detection of inorganic cations in a mixture had already been developed in the second half of the 18th century. After the introduction, in the first half of the 19th century, of organic chemistry as a separate province of chemistry, it was mainly the organic chemists who concerned themselves with qualitative and quantitative organic chemistry. With some notable exceptions, this situation still prevails at the present day. In many American and European universities organic analysis is being taught by the organic chemists. A hybrid position has been and is still being occupied by pharmaceutical chemists, who in the past have contributed materially to analytical chemistry in general.

In the past century and even at the beginning of this century, analytical chemists made use mainly of simple chemical and physical properties, based upon precipitation, colour or gas formation, and visual photometry. The work was mainly empirical in nature, with regard to both the perfection of existing methods and the discovery of new methods.

In the early stages of the development of any field of science, it is necessary to obtain factual knowledge and to make empirical discoveries. The next stage is the interpretation of these facts. As long as a subject is practiced in a purely empirical way it is not yet a legitimate field of science and is looked down upon by other branches of science. We all recall the "cookbook" texts in quantitative analysis. The empirical nature of analytical chemistry in the 19th century and the beginning of this century accounts for the low standing and appreciation of analytical chemistry among the other fields of chemistry. Some of us have devoted our lifetimes to an improvement of this situation and, I am glad to state, with growing success.

Analytical chemistry is based upon the ultimate observation (qualitative) and measurement (quantitative analysis) of a property. Intelligent analytical use of a property requires a full understanding of this property. Let us illustrate this statement briefly by considering the scientific nature of quantitative precipitation and its application to gravimetric analysis.

It is an empirical fact that barium sulphate is a *slightly* soluble substance. The classical gravimetric determination of sulphate or of barium is based upon this *property*. Naturally, the analytical chemist must have a full understanding of all the factors which affect the solubility. Thus, he must be acquainted with the concept of solubility product and with the mass action law in general. We know now that concentrations in equations governed by the mass action law must be expressed in terms of active concentrations or activities. When dealing with an ionic compound like barium sulphate, it is necessary, therefore, to have some knowledge of the effect of ionic strength on the activity coefficient of an ion of given charge. This allows us to predict in a semiquantitative way the effect of ionic strength on solubility. Ionic strength is not the only factor which affects solubility of an ionic precipitate at a given temperature. In addition there are specific effects which can be predicted and even calculated. The solubility of barium sulphate in a solution of hydrochloric acid is considerably greater than in a solution of sodium chloride of the same concentration. Knowledge of the second dissociation constant of sulphuric acid allows us to calculate the specific effect of hydrogen ions on the solubility of barium sulphate. Ions or compounds which form a complex with barium increase the solubility of barium sulphate and again it is possible to calculate the effect quantitatively, provided stability constants of the complexes are known. In the old days of teaching quantitative analysis, an empirical knowledge of the substances which affect the solubility of barium sulphate was taught. But nowadays we require the student to derive in a logical way which types of substances affect the solubility of a slightly soluble ionic compound and even to calculate the magnitude of the effect.

Above we considered only the effect of solubility on the accuracy of a determination. Another important factor is *purity* of the precipitate. This leads us to the subject of coprecipitation. which is still so poorly understood. Coprecipitation involves the carrying down of foreign constitutents with the precipitate. The kind and amount of coprecipitation usually depend greatly upon the conditions during the precipitation. For a scientific approach of a study of coprecipitation it is necessary to have some understanding of the factors which determine the rate of formation of nuclei, the rate of growth of crystallites, the effect of foreign substances upon both rates, adsorptive properties of ionic lattices, rate of adsorption and desorption, laws of solid solution formation, and finally of aging of precipitates.

Our scientific knowledge of the entire subject is still in its embryonic stage. What the analytical chemist wants is a readily filterable precipitate with minimum contamination. The best conditions of precipitation to accomplish this are still found empirically. The entire problem is a typical example of an extensive research topic in quantitative analysis. It requires a systematic scientific study of precipitate formation and purity of precipitates. The objection may be raised that such studies are the domain of the physical chemist or the inorganic chemist or the geochemist or even the biochemist. No doubt they are, and I personally would welcome a clarification of the whole subject by any of these groups of chemists. The point which I would like to state emphatically is that the topic rightfully belongs to the research domain of the analytical chemist. Any information on the subject by other groups of chemists is welcome to us, but so are contributions made by our group to them. Analytical chemistry is no longer the servant of other branches of chemistry, but it is a scientific province of the large field of chemistry. I do not think that we ever should be afraid of tackling a research subject in analytical chemistry because it might overlap with another field. The introduction of a host of organic complex formers and their preparation has been the product of the work of the physical-inorganic chemist, G. Schwarzenbach, who now holds the chair of analytical chemistry at the Eidgenössische Technische Hochschule in Zürich as the successor of W. D. Treadwell. Studies of reactive groups in organic compounds legitimately belong to the domain of the analytical chemist, although any contribution by organic chemists will be highly welcomed by our group. I could go on and on and on. The point is that we make use of the measurement of properties, be they chemical, physical, or both, or biochemical. An intelligent analytical application requires a thorough understanding of the characteristics of the property (like emission and absorption spectra, X-ray spectroscopy, activation analyses, electrochemistry, nuclear magnetic resonance, etc., etc.), the principles of its measurements and finally the technique itself.

A modern education in analytical chemistry requires acquaintance with properties and the principles of their measurement. The demands of a modern education in analytical chemistry are very high considering the ever-increasing number of atomic and molecular properties which are being discovered. Only by providing this broad education in teaching and research can the analytical chemist continue to be recognized as a full-fledged scientific member of the academic and industrial community.

So far I have only concentrated on academic analytical chemistry. Time does not permit a discussion of the role of the analytical chemist in industry. Let me only state that the role of the analytical chemist in industry is quite different now from what it was some twenty-five years ago. Instead of being required to act solely as an analytical technician he now serves and is being recognized as a member of a team composed of chemists and other scientists which is entrusted with the problems which arise in the manufacturing and shipment of new and old products.

Let me assure you that I appreciate the opportunity to make these opening remarks before this Analytical Congress in a country admired by the world at large for its pioneer work and spiri and for its contributions to science and humanity.

In conclusion I would like to express the wish and the confidence that you analytical chemists of Israel will continue to contribute your share to the science of analytical chemistry.

Mrs. Miriam Moses and Dr. Aldo Cantoni discussed the analytical problems at Electrochemical Industries (Frutarom) Ltd., Haifa, which started production in April 1956. The following finished products require analytical control: caustic soda, chlorine, hydrogen, sodium hypochlorite, hydrochloric acid, trichloroethylene, benzene hexachloride, lindane, chloroparaffins, sulphur and various agricultural insecticides. Also, all raw and intermediate products, as well as water, steam, cooling brine and sewage must be checked. The raw materials used in a workshop for ebonite and soft rubber lining of equipment must also be controlled for purity and specifications.

In order to cope with a considerable amount of analytical work, more than 80% of the personnel running the various plants have been trained to run all routine analytical tests, such as gas analyses, titrations, distillations, *p*H determinations, cloud points, etc. The three analysts working in the laboratory are thus left with the more complicated tests requiring instrumentation and perform random checks of results obtained by the operators in the various plants.

All tests are clearly described in written instructions, and for all products standards of purity have been worked out. Shipments of finished products leave the plant only after written approval from the laboratory.

For the two following analytical problems no satisfactory tests have yet been found:

1. *Moisture in dried chlorine.* — All chlorine to be liquefied is dried to a moisture content of not more than 100–150 mg water per cubic meter of air, by scrubbing it with concentrated sulphuric acid. Samples of dried chlorine contain a very fine mist of sulphuric acid and traces of sodium chloride and iron sulphate, which pass through any conventional filtering device. The moisture test is done by passing a known amount of chlorine gas through an absorbing train containing P_2O_5 and measuring its increase of weight. Glass wool is used to filter the chlorine previous to its entering the absorbing train and an additional P_2O_5 U-tube is attached to the end of the train to avoid any possible backward absorption of moisture. Erratic results are obtained due to carry-over of sulphuric acid and of solid powder. A dew-point test was tried but again the presence of sulphuric acid droplets marred the tests. Silica gel was substituted for P_2O_5 but its swelling properties caused occlusion of the absorbing tubes and filtration to test for sulphate was impracticable. The use of a Karl Fischer test is ruled out because of the chlorine.

2. *Moisture in tetrachloroethane.* — In the trichloroethylene plant dry acetylene and chlorine are reacted in tetrachlorcethane which must be dry. It contains dissolved chlorine, some HCl and ferric chloride. Any presence of moisture is now tested by distilling 100 ml of tetrachloroethane and observing how many, if any, of the first distilled drops are turbid. Here again no Karl Fischer test can be made because of the presence of chlorine, and the otherwise quite useful determination of the dieletric constant of the liquid cannot give results because of the varying composition of the liquid tested.

A special lecture on "Voltametry and Amperometric Filtrations with Rotated Electrodes" was delivered at one of the sessions by Prof. Kolthoff in his very clear and lucid manner. A short discussion followed, after which Prof. Kolthoff was presented with a scroll making him the first honorary member of the Analytical Section of the Israel Chemical Society.

LETTER TO THE EDITOR

On the oleoropein from *Olea europaea*. Baruch Shasha and Jeshayahu Leibowitz, *Department of Biological Chemistry, The Hebrew University of Jerusalem.*

The bitter principle of green olives has been described[1] as an ill-defined glucoside oleoropein, allegedly a derivative of caffeic acid. We have succeeded in isolating the compound from the fruit, leaves, roots and stem of the olive tree as a colourless substance, soluble in water, methanol, ethanol, acetone, non-soluble in benzene, chloroform, ether. Its purity has been demonstrated by means of chromatography and electrophoresis. The oleoropein has the composition $C_{24}H_{34}O_{12}$. It reduces Fehling solution and ammoniacal silver solution, gives a positive $FeCl_3$ test, is strongly levorotatory $\alpha_D = -145°$, and is intensely bitter. Crystalline derivatives (tetraacetate, tetrabenzoate and tosylate) have been prepared. The infra-red absorption spectrum of the compound indicates the presence of a double bond, an aromatic nucleus, hydroxyl groups, a ketonic group and an ester bond. This ast feature is intensified in the derivatives. Oleoropein is hydrolyzed by acid and—surprisingly—also by alkali, thus confirming the presence of an ester bond; it is not hydrolyzed by emulsin.

The sugar compound has been identified as glucose. The aglycon consists of two components, one of which has been identified as protocatechuic acid; the other—which in itself shows optical activity—is a new unsaturated acid, $C_{10}H_{16}O_3$, and is now under investigation.

Oleoropein is, therefore, not a glucoside but a double organic ester of glucose. It is oxidized by H_2O_2 and also slowly by free oxygen. It loses its bitter taste on oxidation.

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BOOK REVIEW

Progress in organic chemistry, Volume IV, Edited by J. W. Cook, Butterworths' Scientific Publications, London, 1958, 256 pp., 50 s.

This new volume in the well-known series maintains the high level of the previous volumes. Four chapters out of a total of six deal with the amazing achievements in some fields of natural products chemistry. In the first chapter, *Naturally Occurring Unsaturated Fatty Acids*, F. D. Gunstone surveys the new methods for the preparation of these compounds—especially the stereospecific reactions of acetylene chemistry and anodic syntheses—the recent developments in our knowledge of the structure of familiar and new acids and the improved techniques for their separation and indentification. Theoretical organic chemistry is represented by the chapter *Free Valency in Conjugated Organic Molecules*, where B. and A. Pullman show that the concept of free valency can be useful in the understanding of the reactivity and physicochemical properties of this class of compounds. Products of very interesting and diverse structures synthesized by fungi, such as methylene quinones, chromenopyrones and *spiro*-coumarones are discussed by W. B. Whalley in the chapter *Oxygn Heterocyclic Fungal Metabolites*. In *Naturally Occurring 2-Arylcyclohexane*-1:3-*Diones* by C. H Hussall, an account is given of the achievements in the elucidation of the structure, chemistry and biosynthesis of a range of biologically active products present in ferns and other plants and in the resin of hops, brilliantly crowned by the synthesis of usnic acid. A. H. Cook and G. Harris survey in *Degradation and Synthesis of Peptides and Proteins* the composition, purification, chemical and enzymic methods of determination and synthesis and biogenesis of these products. Methods of synthesis in a specific group of compounds are given in *Heterocyclic Derivatives of Phosphorus, Arsenic and Antimony* by F.G. Mann. The whole volume is stimulating and useful to the organic chemist, especially in view of the difficulty in these times in following directly the advances in all fields of organic chemistry.

J. Klein
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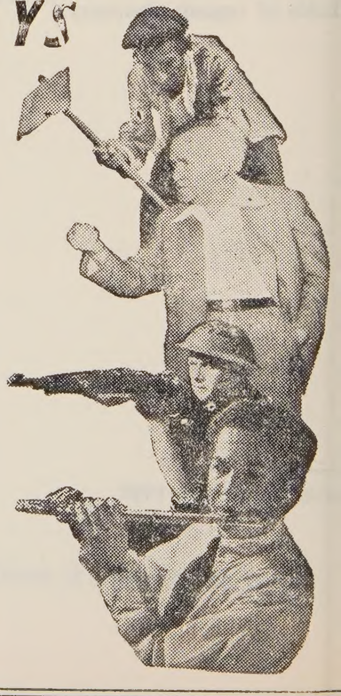

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